A Summary of Current Program 7/1/64

and Preliminary Report of Progress

for 7/1/63 to 6/30/64

EASTERN UTILIZATION RESEARCH AND

DEVELOPMENT DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1963, and June 30, 1964. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Philadelphia, Pennsylvania 19118.

UNITED STATES DEPARTMENT OF AGRICULTURE
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INTRODUCTION

The mission of the Eastern Utilization Research and Development Division is the development of new and improved products and processes based on designated farm commodities, so as to create new and expanded markets for these commodities. The Division conducts research on dairy products, meat, animal fats, hides, tobacco, maple sirup, honey, and Eastern fruits and vegetables, including potatoes, and new crops.

In carrying out its mission, the Division does research in physical and biological science and engineering throughout the spectrum of basic research, applied research and pilot plant development. Division scientists are aware of the key role that basic research plays in uncovering new information that may be later exploited in applied research and development. Hence, a substantial portion of the Division's effort is in basic research.

The Eastern Division has a total staff of about 460, including 226 professional research scientists. The Division is organized in 10 laboratories, of which 7 are located at the Eastern Regional Research Laboratory, Wyndmoor, Pa., one is at Beltsville, Md., with part of its research program at Wyndmoor, and two are at Washington, D. C., with one of the latter, the Dairy Products Laboratory, conducting part of its research program at Beltsville. Two of the Division's laboratories are devoted to pioneering research, one in animal proteins and the other research on allergens found in certain agricultural products.

In addition to research in the Division's own laboratories, contract and grant research supported by the Division and equivalent to about 25 professional man-years per year is going forward at 23 locations in the U. S. The Division's program is supplemented by a variety of research projects in foreign countries under P.L. 480 grants.

In every phase of their research, Division scientists cooperate with representatives of colleges and universities, State Experiment Stations, research institutes and associations, industrial organizations and with other government agencies. Much of the cooperation is informal, but some work is conducted under conditions described in written cooperative agreements and memorandums of understanding. Currently 4 such agreements are in effect.

The farm products with which the Eastern Division deals provide more than half of the nation's cash farm receipts. The major part of U. S. farmland suitable for cultivation is used to provide feed for livestock and dairy cattle; in seven states tobacco provides more cash receipts than any other field crop. Hence it is clear that maintaining and enlarging the markets for these and the other farm products under study at the Eastern Division should be a major national concern.

The opportunities are great. A striking illustration is provided by meat and milk; a one percent increase in meat consumption, which might be achieved through improved quality, lower processing costs and new product development, would result in an increase in feed use equivalent to 80 million bushels of corn. Similarly, a one percent increase in milk consumption would result in an increase in feed use equivalent to 25 million bushels of corn.

Division scientists have already achieved much both in terms of discoveries now commercialized and discoveries of a fundamental nature that will be exploited in the future. Some recent examples of progress are as follows:

New Method for Making Dried Whey in Commercial Use. The foam-spray method for drying cheese whey and other dairy products developed by Division scientists is now being used by several large manufacturers of cottage cheese to make dry whey for food use. One large company alone can produce 3 million pounds of dry whey annually. The novel feature of the new process is injection of air into concentrated whey immediately before it enters the atomizer in the spray-drying chamber. The resulting dry product is free-flowing and disperses readily in contrast to dry whey produced by conventional spray-drying methods, which tends to be sticky and lumpy and does not reconstitute readily. Industry has found the new type drying equipment relatively inexpensive to install and operate. The entire cottage cheese industry has a potential to produce nearly a billion pounds of dry whey annually. Since most of the cottage cheese whey production has been disposed as a waste material because of lack of a profitable outlet for it, this development represents a large potential for increased income to the dairy industry, and at the same time it tends to alleviate the problem of stream pollution by dairy wastes.

Biodegradable Detergents from Tallow. Recent investigations by Division chemists indicate that detergents prepared from tallow are biodegradable. Included among the tallow-based detergents tested were the alpha-sulfo acids and their esters and tallow alcohol sulfates and their modifications. Activated sludge digestion tests have shown these to be more easily biodegradable than either branched-chain or linear alkyl benzenesulfonates (ABS and LAS). From companion Division research, it is known that alpha-sulfo acids prepared from tallow are effective lime soap dispersing agents. For these reasons, such compounds may prove useful in soap-detergent combinations. This research also has shown that certain esters of the alpha-sulfo acids are especially effective as wetting agents. Since they compare in effectiveness with the best known commercial wetting agents and can be made very cheaply, commercial interest in them seems assured. Manufacture of alpha-sulfo acids and their esters is being undertaken by several commercial concerns. In the meantime, research is being done to develop more accurate means for evaluating biodegradability necessary as guides for development of detergents that are more easily biodegraded.

New Dehydrated Vegetable Product Produced Commercially. The explosive puffing process developed by Division engineers for preparing dehydrated vegetables capable of reconstitution in from one-fifth to one-tenth the

usual time has been commercialized. A large manufacturer of dehydrated vegetables in California is now selling 3/8-inch carrot dice made by this new explosive puffing process. This product can be reconstituted by simmering for 5 minutes instead of the usual 40 minutes required for conventionally dried pieces of the same size. According to the manufacturer, "Recent evaluations of 'pilot runs' made in 1962 and production runs made in 1963 have shown that development of certain flavors and aromas that are characteristic of aging in conventionally dried carrots are greatly reduced in the 'Puffed Dried' (explosion puffed) carrots." Also, explosive puffing permits making larger dehydrated pieces than is feasible by conventional hot air drying. The process is extending the use of vegetables in dehydrated soups, stews and other products for civilian and military use.

Improved Low-Fat Ripened Cheese Developed. A new method for making low-fat ripened cheese, a product much desired by diet-conscious consumers, has been developed as a result of research of this Division. Heretofore, skim milk cheese of quality suitable for direct consumption has not been available. While some cheese of this type is being produced by conventional methods, it is tough and lacks flavor and is used mostly for processing with other foods. The cheese produced by the new method is relatively soft, mellow, and mild flavored. While somewhat resembling Cheddar cheese, it has only about 5% butterfat in contrast to 31% for Cheddar and contains larger amounts of non-fat milk solids and moisture than Cheddar. The striking improvement in body, texture, and flavor has been accomplished through addition of selected monoglycerides to the skim milk and careful control of operating conditions during processing and curing of the cheese. The "new" cheese should satisfy a sizable consumer demand and provide an appreciable outlet for non-fat milk solids.

It is evident from these examples that the Eastern Division can make highly valuable contributions to agriculture. Indeed, it has been estimated that for utilization research as a whole--adding together the contributions of the Eastern, Northern,Southern and Western Utilization Research and Development Divisions--more than $2.5 billion has been added to the value of products made as a result of the product or process developments of utilization research. This addition represents almost $15 worth of benefits for every dollar spent on utilization research and development in the Department. Each succeeding year has given more return per year for the total research expenditure. During the past 5 years, for example, the benefit-to-cost ratio has increased to 25 to 1, and this increasing rate of return is expected to continue.

AREA NO. 1 AND AREA NO. 2. DAIRY PRODUCTS - CHEMICAL, PHYSICAL AND BACTERIOLOGICAL CHARACTERISTICS: DEVELOPMENT OF NEW AND IMPROVED PRODUCTS AND PROCESSING METHODS

Problem. Dairying is one of the largest segments of American agriculture: dairy products represent about 13 percent of all farm cash receipts; milk production requires 140 billion feed units annually (1 unit is equivalent in feed value to 1 bushel of shelled corn); milk is a highly nutritious food. It is clear from these facts that research which succeeds in increasing the consumption of milk will have far-ranging effects in raising nutritional levels, in increasing farmers' income, and in increasing consumption of feeds. There is opportunity to increase milk consumption, for per capita consumption is currently at its lowest point in over 30 years at 603 pounds, whole milk equivalent. Current consumption in the U. S. is well below that of several foreign nations, including New Zealand, Canada, Australia, Sweden, Norway and the United Kingdom, all using more than 800 pounds per capita.

Increased consumption can result from improved quality of manufactured dairy products, from cost reductions based on improved processing technology, from the development of new products, or from any combination of these. The develment of new and improved processes and products is the objective of utilization research on dairy products.

Both basic and applied research in this field are needed; applied research is the direct antecedent to the development of new products and processes and basic research provides the information which permits applied research to proceed most effectively.

Increased emphasis on basic research has been advocated by the Animal and Animal Products Research and Marketing Advisory Committee, and the National Agricultural Research Advisory Committee. Basic research is considered primarily the responsibility of public agencies which disseminate their findings for use by all.

One aspect of the problem posed by dairy products is the great need for fundamental information on the complex biophysical-chemical system which each dairy product is. The development of new products and new processing technology through applied research represents the exploitation of fundamental information. Such exploitation and development cannot continue indefinitely; the supply of fundamental information must be maintained and enlarged, and this is the purpose of basic research. The complexity of milk makes necessary the employment of several scientific disciplines in basic research on this commodity. These disciplines undertake investigations needed to identify and measure the amounts of individual chemical components present. They also seek to define the molecular structure of these components; how the molecules react; and the forces determining the course of the reactions. These studies should be intensified. Other needed investigations include: study

of the synthesis of milk; the properties of milk fat; and the factors responsible for the flavor of dairy products and the changes in flavor which occur during processing and storage.

There is also need for a vigorous and sustained program of applied research which is aimed to increase consumption of dairy products. Such a research program could stimulate consumption by development of products with increased palatability, convenience, or extended shelf life. Another opportunity is the possibility of developing new and improved processing technology which wi will reduce costs. Because the price elasticity of milk and milk products greater than that of most food commodities, cost reduction is an attractive avenue for increasing consumption.

Still another opportunity is the development of new milk products of low fat content, for example, a low-fat Cheddar type cheese. Such a development could alleviate problems posed by current controversy over the effect of animal fats in the diet and should provide a product attractive to diet-conscious people.

Increased milk consumption, however achieved, should have a powerful upward effect on feed consumption. Since it is estimated that milk production requires 140 billion feed units annually, a 1 percent increase in milk production would require feed equivalent to 25 million bushels of corn--the production of some 500,000 acres. If the feed were supplied by cropland pasture, more than a million acres would be needed.

It is thus manifest that utilization research leading to dairy product and process development can provide a powerful stimulus to American agriculture.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving chemists, biochemists, microbiologists, food technologists, and engineers, engaged in basic research on the composition and properties of milk, and in applied research directed to the development of new and improved dairy products and processing technology.

The Department's research facilities are located in Wyndmoor, Pennsylvania, Washington, D. C., and Beltsville, Maryland. The Federal scientific effort devoted to research in this area totals 91.9 professional man-years (p.m.y.), which includes 12.3 p.m.y. in the domestic contract and grant research program. The effort is distributed as follows:

(a) Work on flavor aspects of dairy products involves 6.0 p.m.y. at Washington. One category of the flavor studies is concerned primarily with oxidized flavor. In this connection, contract research at Oregon State University, Corvallis, deals with methodology for evaluating flavor of concentrated milks; 0.4 p.m.y. is involved. A second category in the flavor investigations deals mainly with stale flavor. In this classification, contract research at the University of Illinois, Urbana, on possible flavor improvement in aged sterile milks was terminated during the year. A third

category in flavor studies relates to flavor of cultured dairy products. In this category, contract research at the Ohio Agricultural Experiment Station, Columbus, on the source of Cheddar cheese flavor was completed during the year. A fourth category in flavor research will be concerned with studies on isolation and identification of constituents responsible for desirable flavors in butter; this is the subject of research supported by a grant at Oregon State University, Corvallis (1.2 p.m.y.). In addition, research sponsored by the Department under P.L. 480 grants is in progress at: (1) National Dairy Research Institute, Karnal, Punjab, India, on sulfur compounds in relation to flavor and stability of milk; (2) Institute of biochemistry, University of Turku, Finland, on growth-promoting factors for lactic acid bacteria in cheese; (3) Biochemical Institute, Helsinki, Finland, on dietary factors controlling flavor in milk. P. L. 480 research at the National Institute for Research in Dairying, University of Reading, England, on microorganisms in dairy products has been completed.

(b) Research on whole milk products involves 21.0 p.m.y. at Washington and Wyndmoor. The program includes fundamental and applied research on development of liquid milk concentrates (6.0 p.m.y.) and dry whole milk (15.0 p.m.y.) that will be acceptable to the consumer market in quality and storage stability. In the category of liquid concentrates, a research grant at Ohio State University, Columbus, (0.8 p.m.y.) is obtaining data on the physical state of calcium phosphate-containing casein micelles in the concentrates. A new research grant at North Carolina State University, Raleigh, (1.0 p.m.y.) will be concerned with the physical changes associated with steam injection and bubble collapse during milk concentration. In addition to the domestic research on whole milk, the Department sponsors the following under P. L.480 grants at: (1) Technical University Berlin, Berlin, West Germany, on surface changes in fat globules of dried whole milk; (2) Centro Experimental del Frio, Madrid, Spain, on protein destabilization in frozen concentrated milk.

(c) Basic research on milk involves 33.6 p.m.y. at Washington and Wyndmoor. These long-range fundamental studies include the following subjects: structures and interactions of casein and other milk proteins; bacterial spores; structure and properties of nucleic acids; influence of genetics on structure of milk proteins (cooperative with the Animal Husbandry Research Division, ARS) heat stability of milks; milk enzymes; relation of diet of the cow to milk composition. Recently initiated contract research at the University of Minnesota, St. Paul, (0.6 p.m.y.) will investigate the heat stability problem, and recently executed contract research at the University of Maryland, College Park, (0.7 p.m.y.) will be involved with the cow diet-milk composition problem. In addition, research sponsored by the Department under P. L. 480 grants is in progress at (1) National Dairy Research Institute, Karnal, Punjab, India, on milk coagulating enzymes; (2) Indian Institute of Science, Bangalore, India, on phosphoproteins of milk; (3) National Dairy Research Institute, Karnal, Punjab, India, on the proteosepeptone fraction of milk; (4) Institute National de la Recherche Agronomique, Paris, France, on non-protein nitrogenous constituents of milk; (5) Institut National de la Recherche Agronomique, Paris, France, on proteolytic activity of rennin on casein; (6) Centre de Recherches sur les Macromolecules, Strasbourg, France,

on structure of nucleic acids in connection with the synthesis of milk proteins; (7) University of Uppsala, Sweden, on methods for purification of protein complexes applicable to milk; (8) National Institute for Research in Dairying, University of Reading, England, on studies on selected enzymes of milk; (9) Instituto Nacional de Tecnologia, Rio de Janeiro, Brazil, on a study of the active site of trypsin with objective to obtain information useful in developing new dairy products.

(d) Research on milk fat, cheese, and nonfat milk involves a total of 12.0 p.m.y. in Washington and Beltsville. A considerable number of lines of work are associated with butter and butteroil stability and flavor. Cross references to these lines of work are provided in the Report of Progress section. With the availability of 7.0 p.m.y. following termination of the radionuclides investigation at Beltsville on June 30, 1964, 4.0 p.m.y. were assigned to milk fat research as of July 1, 1964. Attempts have been made to locate a suitable contractor to carry out research on fractionation of milk fat for specific food uses; implementation will depend on allocation of funds for contracting.

Research on improved ripened cheese has involved 3.0 p.m.y. during the past year at Washington. Following closeout of the radionuclides work on June 30, 1964, 3.0 p.m.y. additional were assigned to cheese research to make a total of of 6.0 p.m.y. on July 1, 1964. Contract research on Cheddar cheese is mentioned under (a)--flavor of cultured dairy products. It is anticipated that contract pilot plant research on low-fat Cheddar type cheese will be initiated in F. Y. 1965, provided funds are allocated. In addition, research sponsored by the Department under P. L. 480 grants is in progress at: (1) Kaira District Cooperative Milk Producers Union, Ltd., Anand, India, on potential use of American export dry nonfat milk in manufacture of buffalo milk cheese; (2) Institute of Dairy Industry, Warsaw, Poland, on increasing vitamin B content in cheese; (3) College of Agriculture in Olsztyn, Poland, on mechanisms in the cheese-ripening process.

Research on nonfat dry milk involves 2.0 p.m.y. at Washington. Contract research at the University of Wisconsin, Madison, (0.3 p.m.y.) is concerned with the effects of nonfat dry milk on bread yeast.

(e) Research on the identification and removal of radionuclides from milk has involved 7.0 p.m.y. at Beltsville during the past year. Work on this project was terminated June 30, 1964, and EU personnel transferred to cheese and milk fat studies. The radioactive isotopes removal studies were conducted cooperatively with the Atomic Energy Commission and the U. S. Public Health Service which provided 2.0 p.m.y. additional. A research contract with the Producers Creamery Company, Springfield, Missouri, is concerned with development of a commercial scale process for removing radioactive contamination from fluid milk. This contract, supported equally by the Eastern Division and the U. S. Public Health Service, involves 7.3 p.m.y. for the USDA effort.

(f) Pioneering research on the allergens of agricultural products involves 7.0 p.m.y. at Washington.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

Research in progress on the composition and basic chemistry of dairy products begins with work designed to determine the effect of ration and season on milk composition. It extends from basic research on milk proteins and lipids to studies dealing with the chemical compounds present in specific dairy p products such as cheese. Information is sought on the physical and chemical characteristics of the various protein components of the fat globule membrane. In work aimed at determining the nature and extent of the natural variation in milk with respect to its general protein components, a protein of low molecular weight and high phosphorus content has been isolated. A major study of the size, shape, structure and interactions of milk proteins has the specific objective of determining effects of protein-protein and protein-solvent interactions and changes due to enzyme action.

In the case of milk lipids, gas-liquid chromatographic techniques are being used to determine variations in and effects of feeds on the fatty acid composition of milk. Other researches attempt to establish the extent and character of solid solutions of milk fat; the influence of physical state and dispersion of the fat on lipase activity; the influence of certain metals on development of oxidized flavor in milk and milk products; and factors determining the action of lipase enzymes, including inhibitory substances.

The microbiological program with milk and dairy products begins with study of the physiology of the various organisms and extends through their application in milk processing. Lactic cultures in current use are characterized with respect to rate and total acid production, biacetyl and CO_2 production, curd tension, protein hydrolysis and contamination with undesirable species and strains. Various environmental and nutritional factors governing the growth of typical organisms are studied in pure culture. The stimulatory effect of peptide-rich extracts for certain lactic acid bacteria are being investigated. In addition, much attention is given to factors of sanitary importance, including potentially pathogenic organisms, in handling milk products.

In studies of the heat induced changes in milk, milk and milk constituents are subjected to controlled heat treatments and the changes related to flavor are characterized. An immuno-electrophoresis method has been adopted for measuring the effect of varying heat treatments upon milk proteins. High-temperature short-time, conventional heat exchange methods and direct steam injection methods of heating are used. Particular attention is being paid to changes associated with flavor development; with flavor deterioration; and with gelation and sedimentation problems in concentrated milk.

A sizeable program of research is directed to isolation, characterization and identification of the volatile and other compounds which are responsible for the desirable and attractive flavor of milk and other dairy products. A portion of this effort is devoted to establishing the chemical nature of certain

off-flavors.

A number of studies seek to improve the quality of dairy products through improved processing procedures or development of new products. Better processing methods are sought for ice cream, dry milk products, butter, cheeses and fluid milk.

The total number of professional man years devoted to this program is 90.3.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Flavor Aspects of Dairy Products.

Investigations have continued on the flavor of milk and other dairy products. Control of oxidized flavor development is believed to have been virtually realized. Attention has recently been focused on overcoming stale flavor development, a major quality defect in sterile concentrated and dry whole milk. Another flavor study in progress is concerned with factors responsible for Cheddar cheese flavor.

1. Oxidized flavor. A line of research on factors affecting flavor and storage stability of foam-dried whole milk was completed on December 31,1963. The effects of five factors on oxidized flavor were evaluated: oxygen level of the dried milk; heat treatment of the milk; added antioxidants; moisture level; and storage temperature. These factors were interrelated and the last four were effective only in the presence of oxygen. Foam-dried milk of acceptable beverage quality over a period of six months and longer was obtained with low-heat products stored at low temperature in a nitrogen atmosphere substantially free of oxygen. Residual oxygen in the pores of foam-spray dried milk powder can be removed with a scavenging system consisting of a catalyst pellet and 5% hydrogen added to the nitrogen pack. In the continuous vacuum foam process, the product is collected and packaged in nitrogen without ever coming in contact with air.

Flavor tests were conducted during the year on 29 sterile milks at 1:1, 2:1 and 3:1 concentrations to compare scores assigned by trained with those given by preference panels. The concentrates were obtained from six manufacturers. Both the Dairy Products Laboratory and the Oregon State University (contractor) trained panel values for total and scorch-defect scores gave rather high correlations with preference scores and could thus likely be used for predictive purposes. The total score and evaluation of scorch defect in sterile milks assigned by trained panels show general agreement with judgment of an untrained preference panel.

Work on encapsulation of milk fat as a potential means of attaining flavor stability was initiated upon the arrival of a new employee. Edible film-forming substances are being used in the attempt to produce membranes of sufficient strength and continuity to preserve milk fat quality over long-term storage and handling. Preliminary results are encouraging.

2. Stale flavor. Stale flavor of milk products is caused by at least two types of compounds--lactones, formed from precursors in the fat, and ketones, some of which are believed to originate from the fat. Stale flavor development does not require oxygen, and it occurs after only a few weeks of storage in all concentrated and dried milks made by conventional manufacturing methods. The chemical nature and origin of stale flavors are still in the early investigative stages. Gamma and delta aliphatic lactones ranging from 10 to 14 carbon atoms in molecular size are generated when milk fat is heated and stored. A series of odd carbon chain-length methyl ketones ranging from C_5 to C_{15} are also generated upon heating milk fat. These lactones and ketones are strong flavor compounds and have organoleptic significance at the parts per million level at which they occur in heated milk.

Formation of methyl ketone in butteroil has been found to be dependent on presence of moisture. Hence, it is essential that all moisture be removed from butteroil to insure storage stability. O-aminoacetophenone is another ketone isolated from dry whole milk; presence of this compound at concentrations around 0.4 p.p.b. was found to cause a grape-like flavor in stale powdered milk.

Contract research completed during the past year at the University of Illinois has demonstrated that high-temperature short-time (HTST) sterilized, aseptically packaged milk is slightly superior in storage stability to in-can sterilized milk. About 14 compounds in the classes of lactones, ketones and carboxylic acids were identified in the sterilized milks.

It has been suggested that the lactone and ketone generating potential of milk fat can be destroyed by vacuum steam-stripping the fat. The idea is that the total potential lactones and ketones are formed during the process and removed in the steam condensate. It was found in EU research that deodorization of butterfat by steam stripping prevented lactone-type flavor development and produced a fat that was essentially as good in flavor after five months' storage at 80° F. as the control fat was after 0° storage. Evaporated milks have been made with deodorized fat and with control fat. Although not acceptable for beverage purposes, the taste panel preferred the evaporated milks prepared from deodorized fat.

O-aminoacetophenone, first identified in stale dry milk as a strong flavor compound originating from the nonfat portion (perhaps from tryptophane), is now suspected of being present also in HTST liquid concentrates. Taste panel studies have been initiated on milk samples deliberately supplied with certain flavor defects by additives, by use of poor processing techniques, and/or employment of poor storage conditions. Flavor defects to be evaluated include: (1) cooked flavor; (2) heated and stored milk-fat flavors (lactones, ketones); and (3) flavors attributed to maltol and O-aminoacetophenone.

Another approach to the problem of eliminating or preventing development of lactone and ketone flavors in processed milk products would be to select milks of low lactone- and ketone-generating potential. The hydroxy and keto acid precursors of these flavor compounds are products of lipid metabolism in the

cow. Analysis of individual cow milks has indicated a wide variation in the quantities of beta keto acids present. The effects of such variables as type of feed (high grain vs. high forage), season, and individual cow metabolism are to be studied in their relationships to levels of the flavor precursors.

Research at the National Dairy Research Institute, Karnal, Punjab, India, on sulfur compounds in relation to flavor and stability of milk, supported by a P. L. 480 grant, is in the preliminary stage without significant progress yet to report. The organizational phases and instrumentation have been receiving attention.

The new research project at the Biochemical Institute, Helsinki, Finland, on dietary factors controlling the flavor of cow's milk, supported by a P. L. 480 grant, is in the preliminary stage and no findings are available yet for reporting.

3. Cultured dairy products. The discovery of the relationship between the concentration of thiamine disulfide reducing substances (TDRS) and the intensity of desired flavor in Cheddar cheese is believed to be an important contribution toward identification of specific flavor-contributing substances. Development of TDRS is adversely affected by heating the milk prior to cheesemaking. Thus, this seems to explain why cheese made from raw or mildly heated milk cures to give a better flavor than that from pasteurized milk. Greater understanding of the source of Cheddar cheese flavor should now make it possible to develop practical methods of flavor control. Work on this contract research at the Ohio Agricultural Experiment Station was completed during the past year.

Research under a P. L. 480 grant at the Institute of Biochemistry, University of Turku, Turku, Finland, is concerned with growth-promoting factors for lactic acid bacteria in cheesemaking. This project is supplying much detailed and specific information on the growth cycles of lactic acid bacteria isolated as pure cultures from various dairy products. Although these microorganisms possess very complex nutritional requirements and several strains demand unknown growth factors, it was possible to formulate a basic synthetic growth medium. This was used as a basis for exploring the antagonistic and stimulating influences of certain chemicals, vitamins, amino acids, and growth regulators. Experiments on Streptococcus thermophilus show that calcium has growth-promoting action, but serine is growth-inhibiting and antagonistic in the presence of several other amino acids. Such information will be of value in deriving maximum benefit from the use of lactic acid starter cultures in cheesemaking.

Work on the P. L. 480 research project at the National Institute for Research in Dairying, University of Reading, England, on microorganisms in dairy products was completed on May 7, 1964. Findings under this investigation of cultured dairy products were reported last year.

B. Whole Milk

1. Liquid milk concentrates. A meeting of the Evaporated Milk Association, polyphosphates manufacturers, FDA and EU representatives was held to discuss the commercial use of polyphosphates as an additive to evaporated milk (2:1 concentrate). With establishment by EU research that polyphosphates are the most effective consistency stabilizers known for HTST sterilized concentrated milk, it has become necessary to know more of the fate of polyphosphates in milk. Hydrolysis of polyphosphate in milk has been found to be different than that previously observed in aqueous media. While 3:1 concentration has been believed to be the upper practical limit, experiments are being made to explore the feasibility of concentrating sterile milk to 4:1. A program is underway to determine the optimum processing conditions for producing 4:1 liquid concentrate that may be stable during storage. A test marketing of commercially produced 3:1 milk concentrate, stabilized with a polyphosphate, is now being conducted by the University of Wisconsin. Polyphosphates are also to be considered as stabilizers for frozen dairy concentrates.

Investigations are in progress on casein micelles and peptide bonds of proteins in relation to stability of dairy concentrates. Grant-supported research at Ohio State University is obtaining data on the physical state of calcium phosphate-containing casein micelles in milk concentrates. Crystalline structures have been found in frozen 3:1 concentrates. Work has been continued on a modest scale in following the course of chemical bond formation, particularly the peptide bond, by measuring the accompanying changes in the attenuation of reflected infrared energy. This technique is being used in studying reversible sol-gel transformation in milk concentrates.

A new research grant at North Carolina State University will be concerned with the physical changes in milk and milk concentrates associated with steam injection and bubble collapse. Steam injection is a promising technique for providing ultra-high temperatures for HTST sterilization, and it now becomes necessary to examine the physical changes accompanying this type of heating. No progress has yet been made toward research findings.

Research is being done at the Centro Experimental del Frio, Madrid, Spain, under P. L. 480 grant on investigating protein destabilization in frozen concentrated milk. Studies on the effects of freezing milk under various conditions on the distribution of lactose, protein and salts in the thawed separated portions have been made. Rapid freezing by spraying milk into cold air was carried out. Much data was obtained but an improved method of making frozen concentrated milk has not been found.

2. Dry whole milk. Further progress has been made in the two pilot plant processes for preparing dry whole milk--foam-spray drying at Washington and continuous vacuum foam drying at Wyndmoor. Both products initially possess good flavor. The foam-spray dried product has greatly improved dispersibility over conventionally spray dried whole milk but surface frothing at time of reconstitution still remains somewhat of a problem. Samples of the foam-spray dried product were well received by a consumer audience at the Food and

Home Fair in Washington in April 1964. While samples of the continuous vacuum foam product have not yet been distributed, very encouraging advances have been made in storage stability and in optimization of processing variables consistent with economy in processing cost.

Further progress has been made in investigating the physico-chemical factors influencing milk fat/plasma emulsion stability. The latter is important in several ways including control of dispersibility of dried whole milk and storage stability of the dry product. The film that forms on the water surface during reconstitution of dry whole milk has been found to contain 70% fat. Data have been obtained on the antioxidant distribution between butter-oil and water at different temperatures; such information should make it possible to use antioxidants more effectively. Work has been initiated on the use of the Coulter Counter for determining particle size-frequency distribution of the fat globules in milk. Such data should be valuable since globule size is probably related to dispersibility and storage stability of dry milk.

(a) Continuous vacuum foam drying. An experimental program in development of the continuous vacuum foam drying was designed to appraise the effects of seasonal changes in milk properties and of 5 of 13 controllable variables on dryer capacity and product quality. The 5 variables first investigated are: milk concentrate viscosity, content of dispersed gas (nitrogen), phospholipids (lecithin) level, belt loading and degree of vacuum. The effects of the additional controllable variables, those representing the drying conditions, are being determined and the results are combined sequentially with results from earlier less complete experiments. Tentative conclusions reached from a single season's experience are applied during the next corresponding season in moving toward optimal operating conditions. The evolutionary development of the complex drying process has become dependent on computer analysis of accumulating data, and the development has been seriously retarded by delays in processing data from finished experimental designs.

Milk powders from the continuous vacuum process have been stored at refrigerator temperature (40° F.) and room temperature (73° F.) for more than a year; storage stability proved to be limited at 73: powders made and packaged with essentially no contact with oxygen stored well at 40° for at least one year. Addition of a phospholipid, e.g. lecithin, is necessary in order to reduce the foam persisting on the belt during the early phase of vacuum drying, when operating with summer milk. Powders made from summer milk with soya lecithin added were found to have the same flavor stability as winter milk powders made without lecithin. Limited observations suggest that there may be a worthwhile gain in flavor stability at 73° if the residual moisture content of the powder is less than 3%. This low level has been attained, without heat damage, through in-package final drying by calcium oxide. The rate of final drying is so slow, however, that this procedure is not entirely satisfactory. In the hope of achieving equivalent drying much faster, the continuous vacuum dryer has been partitioned so that the milk foam is exposed to an atmosphere of very low humidity during the last one-third of its travel on the dryer belt. The consequences of this modification are now being determined.

The laboratory investigation of milk concentrate foam stability is now being integrated more closely with the continuous drying studies on the hypothesis that foam stability as measured in laboratory tests might be used to specify a quantitative "seasonal" parameter for fluid whole milk. In this manner seasonal changes could be considered in the experimental designs as an uncontrolled but measurable variable. Milk used in the continuous drying process is sampled, concentrated and the concentrate tested for its foaming characteristics. The rate of foam subsidence thus specified is treated as an independent variable.

(b) Foam-spray drying. Research findings on factors affecting the flavor and storage stability of foam-spray dried milk were summarized earlier in this report under "Flavor Aspects of Dairy Products." Concentrated milk foamed by injection of air, nitrogen or CO_2 before spray drying yields a product of excellent flavor. Injection of liquid CO_2 into the high pressure feed line of a conventional spray dryer produced distinctive powder particles that have a very open structure and produce little frothing on reconstitution in contrast to the dried product from nitrogen foaming. The spontaneous dispersion or sinking property of the powder in water has been improved by use of CO_2 foaming but not yet to the desired level. The solubility of milk powder made by CO_2 foaming is somewhat lower than that made with air or nitrogen. It is apparent that the choice of gas used in the foam-spray dryer can radically alter the structure of the powder particles. Some study has been made of ease of reconstitution of milk powders containing additives intended to enhance wettability and sinkability. Preliminary data indicate that surface-active agents may actually have a greater influence on the solubilization of the milk protein than on the dispersion of the milk fat during reconstitution.

A method was devised to fractionate butteroil by continuous crystallization from cold acetone. The primary object is to improve the ease of dispersion of milk powders by using only the more fluid fraction instead of the entire milk fat in making the powders. A possible additional result to be looked for is the concentration of flavor (or off-flavor) precursors and flavor stabilizers in the fractions. The acetone fractionation method is simple and could be scaled up easily. It has yielded a fraction, which contains 80% of the butteroil triglycerides, that is liquid at 68° F. The unsaturated and the low molecular weight fatty acid residues are concentrated in the liquid fraction. The cholesterol content apparently can be reduced by inclusion of an additional step in the fractionation.

In addition to devising means for reducing the foaming on reconstitution, a second engineering approach to improved dry milk has been to attain better fat dispersion by homogenizing the concentrate in the high pressure line between pump and spray. Homogenization of the product at the high solids concentrations now being used was previously considered impossible but was recently achieved by use of an homogenization valve. Use of this equipment leads to a marked reduction in the amount of "free" fat in the finished powder. It results in lower processing cost and should also make commercially feasible a method of manufacturing a relatively high fat-containing powder from recombined nonfat milk and modified milk fat. There is considerable interest in

the combination of butteroil and nonfat milk for providing fluid beverage milk for human feeding under the foreign aid programs and perhaps elsewhere.

One thousand cans of freshly prepared foam spray dried whole milk were distributed to visitors at the Department's Food and Home Fair in April. Each can of milk powder was the equivalent of about 2/3 quart of milk. An evaluation card was provided with each can. The proportion of cards returned, 15 percent, was normal for this sort of consumer reaction test. Ninety-five percent of the respondents indicated that the flavor is acceptable; Eighty-eight percent were satisfied with the way the milk powder reconstitutes in cold water, although some objected to its tendency to foam.

A comparison of the keeping quality of milk powders packaged in tin cans and in flexible plastic pouches has been made. The same hydrogen plus catalyst oxygen-scavenging capability was provided in both types of containers. In the cans "low heat" milk powders stored at 40° F. have maintained flavor scores well within the range of acceptability during the 4 months' duration of the test to date. None of the plastic containers tested thus far have been sufficiently gas-tight to permit comparable keeping quality. At 80° F. the flavor scores of milk powders packaged both in cans and in pouches decreased into the nonacceptable range within two months.

Studies are being carried out on surface changes in fat globules of dried whole milk under P. L. 480 grant at the Technical University Berlin, Berlin, West Germany. Results obtained using model systems indicated that the products of the amino acid-aldehyde and protein-aldehyde interactions could be isolated and characterized using chromatographic procedures. This work together with results obtained in an extensive study of the reaction of thiobarbituric acid with carbonyls is providing a solid basis for the further study of the interactions occurring on the surface of the fat globules found in milk and dairy products.

C. Basic Research on Milk

1. Isolation of milk proteins. Improved methods have been developed for the preparation of the iron-binding proteins of milk--namely, the red protein, blood transferrin, and lactoperoxidase. The methods involved chromatography on DEAE and phosphocellulose. It was demonstrated by gel electrophoresis that the transferrin of milk and bovine blood are the same.

A modified acrylamide gel electrophoresis was developed and applied to the identification and analysis of milk protein fractions. This method is of particular value in determining the purity of milk proteins.

2. Characterization and structure of milk proteins. Two of the genetic variants of β-lactoglobulin, namely, A and B, as well as the AB form and synthetic mixtures of the two, were characterized by their solubilities in water and dilute sodium chloride. It was found that the B form was about five times more soluble than the A form and that the solubilities of samples of β-lactoglobulin from individual cow's milk of a given type do not vary significantly. Synthetic mixtures of A and B types were found to interact in all proportions giving rise to a mixed crystal form with solubility characteristics of a solid solution which was related to an average of the whey fraction of the

components. Despite the fact that the B form is five times more soluble than the A form, the ratios of the solubilities in salt solution divided by their respective solubilities in water are the same for both forms as well as mixtures of the two. This indicates that the distribution of charges, as well as the amino acid sequences, of the two forms are essentially the same.

Studies on the primary structure of α-lactalbumin were continued and the complete sequences of four larger peptic peptides were established. Work is in progress on several of the other peptides.

Photooxidation studies, which proved to be so useful for the establishment of the active sites of the hydrolytic enzymes, were resumed both on model substances and on insulin. Studies on the photooxidation of amino acids have shown that the reaction proceeds as a true enzymatic reaction if one applies various kinetic treatments as used for enzymatic reactions. It would appear that photooxidation of amino acids or proteins represents an excellent model for enzymatic reactions. Photooxidation of amino acids and proteins with methylene blue was made more specific by lowering the temperature and pH during irradiation. Thus, photooxidation of insulin under such conditions brought about chemical changes, which were confined entirely to the two histidine residues of the β-chain of insulin. Biological assays of the irradiated insultin indicate that the histidine residues are intimately associated with the hormonal activity.

Investigation of the biochemical properties of casein has continued along several lines. Attention has been turned from photooxidation of α_s - and β-caseins to corresponding studies on kappa-casein. Intensive research is in progress to determine the nature of kappa-casein. Genetic studies of variations in α_s- and β-caseins are now virtually complete. Studies have begun on the fat that precipitates with the casein in acid coagulation of milk. Information now being obtained may prove to be of value in accounting for breed, herd and geographical differences in the processing of milk.

3. Interactions of milk proteins and other components. Study of the interactions of milk proteins in solution has been continued with emphasis on: (1) β-lactoglobulin tetramerization; (2) characterization of variant C of β-lactoglobulin and comparison with variants A and B; (3) conformation of whole casein and the α, β and kappa fractions. Accumulation of such basic data is required if the dairy industry is to achieve eventually maximum control of milk protein behavior in processed products.

Investigation of the state of casein in whole milk concentrates has been continued with emphasis on the effects of concentration and sterilizing temperatures on the binding of calcium and phosphate by the caseinate system. When more information is available in this field, processing of improved sterilized and concentrated milk products will be facilitated.

A research contract on the reactions involved in governing heat stability of individual milks has recently been executed with the University of Minnesota. No results are yet available.

4. Heat-resistant spores. The fundamental studies on the chemistry of bacterial spores have led to cultivation methods that yield large synchronous crops of sporulating Bacillus cereus. The sporulating cells are being used in preparing dipicolinic acid-synthesizing cell extracts. Basic information on cell-free biosynthesis of dipicolinic acid should contribute to a better understanding of enzymatic activities of spores in relation to their heat resistance. This in turn may enable the processing industry to sterilize dairy products with less severe heat treatment.

5. Nucleic acids. A fundamental study of the structure, degradation, and other properties and characteristics of ribosomal nucleic acids is in a preliminary stage. RNA is a key component in the protein synthesis mechanism and greater knowledge on this subject will be of importance in genetics. Mammary gland tissue for preparation of RNA is to be supplied by the Dairy Cattle Research Branch.

6. Milk enzymes. Studies on the enzymes in milk have been continued, with emphasis on the ribonucleases. It now appears that there are three ribonucleases, designated A, B and C. Determinations of amino acid composition, carbohydrate components and structure are being carried out to obtain basic information on the ribonucleases. This type of study is also being conducted on the β-caseins, A, B and C and α_s- caseins A, B and C. Hydrolysis of kappa-casein by rennin is yielding interesting results. It is important to know more about the milk enzymes and how they affect milk components during processing.

7. Relation of the cow's diet to milk composition and flavor. A research contract at the University of Maryland on the relation of milk composition, particularly the fat content, to diet of the cow is being negotiated.

8. Basic research under P. L. 480 grant program. Fundamental studies are underway at the National Dairy Research Institute, Karnal, Punjab, India, under P. L. 480 grant on the isolation and use of milk coagulating enzymes of microbiological origin for cheese manufacture. The relative milk-clotting and proteolytic activities of selected bacteria have been determined in a search for a suitable microbial source for a substitute for rennet.

Research is in progress at the Indian Institute of Science, Bangalore, India, under a P. L. 480 grant on structural studies on phosphoproteins in milk and their behavior during processing. This project has not been in effect for any great length of time and no progress report has yet been received.

Investigations are being made on the proteose-peptone fraction of milk under P. L. 480 grant at the National Dairy Research Institute, Karnal, Punjab, India. A new colorimetric method has been devised to determine the proteose-peptone content. Using this method 150 samples of cow milk and 50 samples of buffalo milk were studied. Results indicate that no striking concentration differences exist between the proteose-peptone fractions of the milks taken from these animals.

Research is being done on nonprotein nitrogenous (NPN) constituents of milk under P. L. 480 grant at the Institut National de la Recherche Agronomique, Paris. Studies concerning the soluble NPN products resulting from the action of rennin on casein were concluded. It was established that more than 80% of this fraction could be accounted for by the peptides split from the kappa-casein. Further studies show that the increase in NPN noted on heating milk arises from the release of materials different from those released by the action of rennin on casein.

Research at the Institut National de la Recherche Agronomique, Paris, France, under a P. L. 480 grant is concerned with investigating the proteolytic activity of rennin on casein. This research is concerned with an elucidation of the enzymatic coagulation of milk to obtain basic information for use in devising improved cheesemaking processes. A pure crystalline form of rennin was reacted with the various protein components known to form the casein moiety. It was shown that the clotting action of rennin on milk depends on a reaction between the enzyme and kappa-casein. In the presence of α_s-casein, the action of rennin on kappa-casein was inhibited. The strong tendency of α_s-casein and kappa-casein to interact is believed to be significant also in the heat stability of milk and in the gelation which occurs in condensed milks on prolonged storage.

Fundamental studies underway at the Centra de Recherches sur les Macromolecules, Strasbourg, France, under P. L. 480 grant are probing the relationship between genetic factors and the synthesis of proteins to help explain why a particular kind or quality of protein is formed in a given species of plant or animal. It is envisioned that results from this and related research will have far-reaching effects on the selective breeding of crops and livestock. Since previous research indicates that structural changes in a protein's deoxyribonucleic acid (DNA) molecule dictates the formation of genetically different proteins, these workers are acquiring detailed chemical and physical data on the molecular sub-units of DNA. Substantial progress has been reported in the initial phases of the project. Techniques have been developed for separating native DNA from artificially denatured material, and for detecting compositional differences. The enzyme, spleen deoxyribonuclease, has been isolated in the pure state for the first time and has been utilized in degrading the DNA molecule into smaller units for further study.

The research at the University of Uppsala in Sweden, under a P. L. 480 grant, on methods for purifying protein complexes that would be applicable to milk is just in its beginning. Hence, there is nothing to report at present.

Studies are underway on selected enzymes of milk under P. L. 480 grant at the National Institute for Research in Dairying (University of Reading), Shinfield, Reading, England. A technique for the rapid and direct recording of small pH changes was developed and used to study the detailed kinetic properties of various enzyme systems found in milk. A study of lipase activity in milk taken from 20 cows at various stages of lactation revealed marked differences in lipase activity of milk drawn from individual cows.

Under a P. L. 480 grant at Instituto National de Technologia, Rio de Janeiro, Brazil, studies on the purification of crystalline trypsin resulted in the separation of several enzymatically active components. Methods for these separations were developed both for analytical as well as for preparative scale. The apparent enzymatic heterogeneity of trypsin might be due to the incipient autolysis of the enzyme. Further characterization of these fracO tions will permit the correlation between change in biological activity and the modification of structure.

D. Milk Fat, Cheese and Nonfat Milk

1. Milk fat. Probably the most important technical problems connected with extending the utilization of butterfat are related to flavor and storage stability. Thus in the foregoing sections of this report in "Report of Progress" dealing with flavor research, research on whole milk products and basic research, many of the lines of investigation deal with butter andbutter-oil. For purpose of cross reference, the following topics are discussed under "Flavor Aspects of Dairy Products": encapsulation of milk fat; lactones and ketones originating from milk fat; deodorization of butterfat by steam stripping. Under "Whole Milk" progress, the following subjects pertinent to the fat component are discussed: antioxidant distribution between butteroil and water; fractionation of butteroil; improved dispersion of butterfat in foam-spray drying of milk. Under "Basic Research on Milk," the new contract research on relationship of butterfat and other milk components to the diet of the cow is mentioned.

Many believe that butter use could be materially extended, particularly by taking advantage of its unique flavor and certain other properties, if fractions were available to provide "tailor-made" products. Hence, attempts have been made to locate a suitable contractor to carry out research on fractionation of milk fat for specific food uses. No work has been started on this project, and implementation will depend on allocation of funds for contracting.

2. Cheese. Pilot plant work in 1959-1960 on methods of improving the quality of Cheddar cheese indicated that flavor enhancement of this cheese may be obtained by controlled lipolysis. This phase of research was stopped July 1, 1960, and personnel transferred to Isotope Removal Investigations. Laboratory research continued and resulted in development of a selective medium for Leuconostoc, which provides a new means for detecting flavor-forming bacteria in lactic starters, cheese and other cultured dairy products. An important discovery, now widely used commercially, was that the destructive growth of bacteriophage in cheese starters can be prevented by phosphate-heat treatment. This latter line of work was discontinued on February 25, 1964.

It is considered by many persons engaged in research and marketing of dairy products that a flavorful cheese of good texture, relatively low in fat and high in protein, should meet with ready demand. Continued progress has been achieved on development of a laboratory procedure for producing low-fat cheese. The new low-fat cheese has only about 5% fat compared to 31% for

ordinary Cheddar. While further laboratory and pilot plant work remain to be done, results are quite promising in regard to the texture and flavor factors relative to these quality attributes in low-fat cheese made by conventional methods. It is anticipated that additional manpower can be assigned to this work after July 1, 1964, and initiation of contract pilot plant research is expected, provided funds are allocated.

Studies are being made at the Kaira District Cooperative Milk Producers Union, Ltd., Anand, India, under P. L. 480 grant on investigations of the addition of nonfat dry milk solids to buffalo milk in the manufacture of hard cheese as a means of expanding overseas markets for dry milk. This research shows that Cheddar-type cheese of satisfactory body and texture can be made from native buffalo whole milk when it is standardized with imported nonfat dry milk, but flavor development is slow and insufficient.

Research is being done at the Institute of Dairy Industry, Warsaw, Poland, under P. L. 480 grant on the development of nutant strains of molds with increased ability to synthesize vitamin B for use in improving the quality of mold-ripened cheese. Penicillium roqueforti has been found to increase the level of riboflavin, niacin, pantothenic acid and biotin in Roequefort cheese during ripening. Some of the induced mutant strains were found to be much more vitaminogenic than others.

Investigations are being carried out at the College of Agriculture in Olsztyn, Olsztyn, Poland, under P. L. 480 grant on the mechanisms of the cheese-ripening process to obtain fundamental information for use in developing methods for manufacturing improved quality cheese. An exhaustive study is being made to determine the products formed during the fermentation of fat, protein and lactose in a variety of cheeses in an attempt to establish the mechanisms of fermentation and possible relation between specific fermentation products and flavor.

3. Nonfat dry milk. Foam-spray dried nonfat milk is now being manufactured by several producers using the EU process. One of these processors has the capacity to turn out a large volume of product, and the industrial pioneers in foam-spray drying are presently engaged in developing outlets for this new type of nonfat dry milk (NFDM). Our work on development of increased food outlets for NFDM has been greatly curtailed since the death of a senior staff member about a year ago. While new product work could not be emphasized, considerable progress has been made on equipment and processing. A practical in-line homogenizing valve arrangement has been developed that will reduce equipment cost in spray drying. Development of an in-line high-solids evaporator will permit foam spray drying of 60% nonfat milk solids concentrate under conditions that are expected to avoid protein denaturation. Equipment has been installed for compressing dried milk into cubes to reduce volume markedly.

In addition to the commercial adaptation of foam-spray drying to NFDM, several large dairy products manufacturers have recently started foam-spray drying cottage cheese whey. One of these companies alone can produce 3 million

pounds of dried whey annually. Air is injected into the concentrated whey immediately before it enters the spray-drying chamber. The resulting product is free-flowing and disperses readily in contrast to conventionally dried whey which tends to be sticky, lumpy and difficult to reconstitute. Most of the cottage cheese whey has been dumped in the past. The entire cottage cheese industry has a potential to produce nearly a billion pounds of dried whey annually, worth about $100 million, provided additional food uses can be developed for the product. It has been demonstrated that foam-spray drying is also advantageous for drying Cheddar cheese, milk chocolate mixes, ice cream mix, malted milk and other dairy products.

Bread has provided an important outlet for NFDM. This was threatened when a large fraction of the bakeries went on a short-time continuous dough mixing procedure in which little or no NFDM can be tolerated because of dough handling and loaf texture problems. The objectives of contract research at the University of Wisconsin are to determine the advantages of NFDM in dough fermentation (besides the obvious contributions to nutrition and flavor) and to search for modified conditions of fermentation and dough handling that will permit the use of NFDM in the new continuous breadmaking process. It has been demonstrated to date that nonfat milk solids greatly stimulate the yeast fermentation.

E. Radionuclides in Milk

Extensive nuclear testing, particularly in 1950-1961, made it advisable to develop practical means for removing radioactive isotopes making their way into milk through consumption of feed exposed to fallout. Laboratory and pilot plant studies on removal of radioactive contamination from milk have demonstrated that commercial scale withdrawal of radioactive strontium and cesium by an ion-exchange fixed bed process is feasible. Iodine-131 can be removed by use of an anion resin column if necessary in time of emergency, but further research is needed on removal of this particular radionuclide. Work on this project was terminated June 30, 1964. The studies on removal of radioactive isotopes have been conducted in cooperation with the Atomic Energy Commission and the United States Public Health Service.

An automatic commercial size plant for removal of Sr-90 from milk has been established under contract at Producers Creamery Company, Springfield, Missouri. Findings from the Beltsville pilot plant are being used as a basis for this commercial scale plant. Start-up of the Springfield plant was originally scheduled for March 15, 1964, but was delayed about 60 days because of slow delivery of certain valves.

Cooperative research with the U. S. Public Health Service has been extended through a memorandum of understanding to include a pilot plant study of a moving resin bed ion exchange system for removing radionuclides from milk. A moving bed contactor is being purchased and will be installed at their Southeastern Radiological Health Laboratories, Montgomery, Alabama.

F. Allergens of Agricultural Products

The objective of the milk allergens research is to identify and characterize the constituents in cow's milk that produce undesirable reactions in infant feeding and to seek practical methods of inactivating these allergens. Special laboratory facilities for a collaborative program with research workers at Jefferson Medical College, Philadelphia, were set up and investigations started. However, this collaborative program was unexpectedly terminated when urban renewal displaced and scattered the families that had been counted on to provide clinical material. Hence, negotiations will have to be developed with another medical collaborator in order to continue this study.

Studies on the immunochemical characterization of ribonuclease of cow's milk and from bovine pancreas have been completed. Ribonuclease isolated from bovine milk by the Milk Properties Laboratory was shown to be serologically identical to pancreatic ribonuclease. This suggests that ribonuclease present in cow's milk originates in the glandular tissue of the pancreas, which is considered to be a finding of considerable fundamental importance.

Castor bean allergens have been under study for the past several years. The castor bean allergen fraction designated as CB-1A is being fractionated and characterized as to antigenic and allergenic components. The immunological relationships of CB-1A, castor bean meal, and castor bean pollen are being determined. CB-1A has been found to be a complex mixture of proteins and polysaccharidic proteins with a common antigenic specificity or, at least, closely related antigenic specificities. Studies are being made on the carbohydrate-free protein mixture isolated from CB-1A and designated as CB-65A in search of structural differences that may explain immunological distinctness. The subject of castor bean allergens is extremely complex, requiring development of precise methodology for elucidation of structure and correlation with immunological behavior.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

Flavor Aspects of Dairy Products

Honkanen, E. and Moisio, T. 1962. On the occurrence of oct-1-en-3-ol in clover plants. Acta. Chem. Scand., 17, p. 588.*

Honkanen, E. and Karvonen, P. 1963. A gas chromatographic method for the determination of volatile flavors from foodstuffs. Acta Chem. Scand. 17, pp. 1357-1362.*

Honkanen, E., Moisio, T., Ohno M. and Hatanaka, A. 1963. Mass spectra of seven isomeric hexen-1-ols. Acta Chem. Scand., 17, pp. 2051-2054.*

Kristoffersen, T. 1963. Measuring thiamine disulfide reducing substances in Cheddar cheese. J. Dairy Sci., 46, pp. 1135-1136.

Matikkala, E. J. and Virtanen, A. I. 1963. New γ-glutamylpeptides isolated from the seeds of chives (Allium schoenoprasum). Acta Chem. Scand., 17, pp. 1799-1801.*

Moisio, T. and Honkanen, E. 1963. A small inlet system for a mass spectrometer. Acta. Chem. Scand., 17, p. 1469.*

Muck, George A., Tobias, Joseph and Whitney, Robert McL. 1963. Flavor of evaporated milk. I. Identification of some compounds obtained by the petroleum ether solvent partitioning technique from aged evaporated milk. J. Dairy Sci., 46, pp. 774-779.

Parks, Owen W., Keeney, Mark and Schwartz, Daniel P. 1963. Carbonyl compounds associated with the off-flavor in spontaneously oxidized milk. J. Dairy Sci., 46, pp. 295-301.

Parks, Owen W., Kenney, Mark, Katz, Ira and Schwartz, Daniel P. 1964. Isolation and characterization of the methyl ketone precursor in butterfat. J. Lipid Research, 5, pp. 232-235.

Saarivirta, M. and Virtanen, A. I. 1963. A method for estimating benzyl isothiocyanate, benzyl thiocyanate, and benzyl nitrile in the crushed moistened seeds of Lepidium sativum. Acta Chem. Scand., 17, pp. 574-578.*

Schwartz, D. P. and Parks, O. W. 1963. Methyl ketones in camembert cheese. J. Dairy Sci., 46, p. 1136.

Schwartz, D. P., and Parks, O. W. 1963. Quantitative analysis of methyl ketones in blue cheese fat. J. Dairy Sci., 46, pp. 989-990.

Schwartz, D. P., Haller, H. S. and Keeney, Mark. 1963. Direct quantitative isolation of monocarbonyl compounds from fats and oils. Anal. Chem., 35, pp. 2191-2194.

Schwartz, Daniel P. 1963. Paper chromatography of organic compounds. Chapter in Handbook of Anal. Chem., edited by Louis Meites.

Schwartz, D. P. and Parks, O. W. 1963. Thin-layer chromatography of carbonyl compounds. Separation of aliphatic carbonyls 2,4-dinitrophenylhydrazones into classes. Microchem. J., 7, pp. 403-406.

Schwartz, Daniel P., Parks, Owen W. and Boyd, E. N. 1963. Methyl ketones in Roquefort cheese. J. Dairy Sci., 46, (12) 1422.

Spare, C. G. and Virtanen, A. I. 1963. On the lachrymatory factor in onion (Allium cepa) vapours and its precursor. Acta Chem. Scand., 17, pp. 641-650.*

Whole Milk

Anderson, H. A., Allen, C., Jr. and Tamsma, A. 1963. Some properties of foam-dried whole milk produced using laboratory scale equipment. J. Dairy Sci., 46, p. 615.

Berlin, Elliott and Pallansch, M. J. 1962. Influence of drying methods on porosity of whole milk powder granules. J. Dairy Sci., 45, p. 657.

Berlin, E., and Pallansch, M. J. 1963. Influence of drying methods on density and porosity of milk powder granules. J. Dairy Sci., 46, pp. 780-784.

Fox, K. K., Holsinger, V. H., Harper, M. K., Howard, N., Pryor, L. S. and Pallansch, M. J. 1963. The measurement of the surface areas of milk powders by a permeability procedure. Food Tech., 17, pp. 805-808.

Harper, M. K., Holsinger, V., Fox, K. K. and Pallansch, M. J. 1963. Factors influencing the instant solubility of milk powders. J. Dairy Sci., 46, pp. 1192-1195.

Kilman, P. and Pallansch, M. J. 1962. Some optical properties of casein-lactose interaction products. J. Dairy Sci., 45, p. 651.

Leviton, Abraham, Anderson, Howard A., Vettel, Harold E. and Vestal, James H. 1963. Retardation of gelation in high temperature-short time sterile milk concentrates with polyphosphates. J. Dairy Sci., 46, pp. 310-319.

Sather, Lois A., Calvin, Lyle D. and Tamsma, A. 1963. Relation of preference panel and trained panel scores on dry whole milk. J. Dairy Sci., 46, p. 1054.

Sinnamon, H. I., Komanowsky, M. and Aceto, N. C. 1964. Effects on product quality of cross-circulation air drying of whole milk foam. J. Dairy Sci., XLVII, pp. 90-92.

Tamsma, A., Mucha, T. J. and Pallansch, M. J. 1963. Factors related to the flavor stability during storage of foam-dried whole milk. III. Effect of antioxidants. J. Dairy Sci., 46, pp. 114-119.

Leviton, A. and Pallansch, M. J. January 28, 1964. Process for preparing sterilized milk concentrates. U. S. Patent 3,119,702.

Basic Research on Milk

Alais, C. and Multon, C. 1963. Étude de la sécrétion d'enzyme coagulant dans la caillette de l'agneau. Annales de Biologie animale, Biochimie, Biophysique, 3 (I), pp. 65-70.*

Berlin, E., Curran, H. R. and Pallansch, M. J. 1963. Physical surface features and chemical density of dry bacterial spores. J. Bacteriol., 86, pp. 1030-1036.

Bernardi, G., Griffe, M. and Appella, E. 1963. Isolation and characterization of spleen acid deoxyribonuclease. Nature, 198, pp. 186-187.*

Bingham, E. W. and Zittle, C. A. 1963. Purification and properties of acid phosphatase in bovine milk. Arch. Biochem. Biophys., 101, pp. 471-477.

Curran, H. R. and Pallansch, M. J. 1963. Incipient germination in heavy suspensions of spores of Bacillus stearothermophilus. J. Bacteriol., 86, pp. 911-918.

Fox, K. K., Holsinger, V. H. and Pallansch, M. J. 1963. Fluorimetry as a method of determining protein content of milk. J. Dairy Sci., 46, pp. 302-309.

Garnier, J., Dumas, B. R. and Gautreau, J. 1962. Examen par chromatographie électrophorèse et immunoélectrophorèse de diverses préparations de caséine. Proc. XVI Inter. Dairy Congress, pp. 655-664.*

Garnier, J. 1963. Étude cinétique d'une protéolyse limitée: Action de la présure sur la caséine kappa. Biochim. et Biophys. Acta, 66, pp. 366-377.*

Granier, J. and Brignon, G. 1963. Étude de l'action de la présure sur la caséine par la méthode titrimétrique a pH constant. Comparaison avec d'autres méthodes. Annales Biol. anim. Bioch. Biophys., 3 (I) pp. 65-70).*

Gordon, W. G., Groves, M. L. and Basch, J. J. 1963. Bovine milk "red protein": amino acid composition and comparison with blood transferrin. Biochemistry 2, pp. 817-820.

Groves, M. L., Basch, J. J. and Gordon, W. G. 1963. Isolation, characterization, and amino acid composition of a new crystalline protein, lactollin, from milk. Biochemistry 2, pp. 814-817.

Gutfreund, H. and Hammond, B. R. 1963. Records of pH changes during enzyme reactions and kinetic studies with yeast hexokinase. Nature, 198, pp. 667-670.*

Jollès, P., Alais, C. and Jollès, J. 1963. Étude de la caséine kappa de vache. Caractérisation de la liason sensible à l'action de la présure. Biochim. et Biophys. Acta, 69, pp. 511-517.*

Kalan, E. B., Greenberg, R., Thompson, M. P. and Pepper, L. 1963. Some chemical characteristics of α_s-caseins. Federation Proceedings 22, p. 2966.

Kiddy, C. A., Thompson, M. P., Johnston, O. and Pepper, L. 1963. The genetics of α_s- and β-caseins. J. Dairy Sci., 46, p. 626.

Kiddy, C. A., Johnston, J. O. and Thompson, M. P. 1964. Genetic polymorphism in caseins of cow's milk. I. Genetic control of α_s-casein variation. J. Dairy Sci., 47, p. 147.

Kiesvaara, M. and Virtanen, A. I. 1963. Synthetic ascorbigen as a source of vitamin C for guinea pigs. Acta Chem. Scand., 17, pp. 849-853.*

Pepper, L. and Thompson, M. P. 1963. Dephosphorylation of α_s- and kappa-caseins and its effect on micelle stability in the kappa-α_s-casein system. J. Dairy Sci., 46, pp. 764-767.

Peterson, R. F. 1963. High resolution of milk proteins obtained by gel electrophoresis. J. Dairy Sci., 46, p. 1136.

Piironen, Eino and Virtanen, Artturi I. 1963. The effect of thiocynate in nutrition on the iodine content of cow's milk. Zeitschrift für Ernährungswissenschaft, 3, pp. 140-147.*

Thompson, M. P. 1963. DEAE-cellulose-urea chromatography of casein. J. Dairy Sci., 46, p. 607.

Thompson, M. P., Pepper, L., Gordon, W. G. and Basch, J. J. 1963. The chemical composition of bovine α_s-A, B and C caseins. J. Dairy Sci., 46, p. 607.

Thompson, M. P. and Pepper, L. 1964. Some characteristics of α_s- and β-casein variants. J. Dairy Sci., 47, p. 293.

Virtanen, A. I. 1963. Production of cow's milk without proteins using urea and ammonium nitrogen as the sources of nitrogen, and purified carbohydrates as the sources of energy. Suomen Kemistilehti, 36, pp. 83-84.*

Virtanen, A. I., Kreula, M. and Kiesvaara, M. 1963. Investigations on the alleged goitrogenic properties of milk. Zeitschrift für Ernährungswissenschaft, Suppl. 3, pp. 23-37.*

Virtanen, A. I. and Wahlroos, Ö. 1963. Absence of 6-methoxybenzoxazolinone in uninjured maize tissue. J. Pharm. Sciences, 52, pp. 713-714.*

Wahlroos, Ö. 1962. A study of gas-liquid multiple distribution with mixing of the liquid phase. Annales Academiae Scientiarum Fennicae, Series A, II Chemica, p. 122.*

Weil, L. and Seibles, T. S. 1964. On the structure of α-lactalbumin. II. Amino acid sequences of four peptides formed in the peptic hydrolysis of the native protein. Arch. Biochem. Biophys., 105.

Witz, J., Timasheff, S. N. and Luzzati, V. 1964. Molecular interactions in β-lactoglobulin. VIII. Small-angle x-ray scattering investigation of the geometry of β-lactoglobulin A tetramerization. J. Am. Chem. Soc. 86, p. 168.

Woychik, J. H. 1964. Investigation of reduced kappa-casein from cow's milk. Federation Proceedings 23, p. 474.

Zittle, C. A. 1963. Photooxidation of α_s-casein. J. Dairy Sci., 46, p. 607.

Zittle, C. A. and Custer, J. H. 1963. Purification and some of the properties of α_s-casein and kappa-casein. J. Dairy Sci., 46, p. 1183.

Zittle, C. A. and Walter, M. 1963. Stabilization of β-casein and kappa-casein against precipitation by calcium chloride. J. Dairy Sci., 46, p. 1189.

Zittle, C. A. 1964. Xanthine oxidase, acid phosphatase, ribonuclease and protease. J. Dairy Sci., 47, p. 202.

Milk Fat, Cheese, and Nonfat Dry Milk

Bell, R. W., Hanrahan, F. P. and Webb, B. H. 1963. Foam spray drying methods of making readily dispersible nonfat dry milk. J. Dairy Sci., 46, pp. 1352-1356.

Kapsalis, J. G., Kristoffersen, T., Gould, I. A. and Betscher, J. J. 1963. Effect of chemical additives on the spreading quality of butter. II. Laboratory and plant churnings. J. Dairy Sci., 46, pp. 107-113.

McDonough, F. E., Hargrove, R. E. and Tittsler, R. P. 1963. Selective planting medium for Leuconostoc in mixed lactic cultures. J. Dairy Sci., 46, pp. 386-390.

Bell, Raymond W. October 29, 1963. Process for producing sweetened condensed cream. U. S. Patent 3,108,875.

Radionuclides in Milk

Easterly, D. G., Harris, J. Y., Bunce, L. A. and Edmondson, L. F. 1963. Factors affecting the removal of radiostrontium from milk by ion exchange resins. J. Dairy Sci., 46, pp. 1207-1210.

Easterly, D. G., Edmondson, L. F., Avants, J. K. and Sadler, A. M. 1964. Effect of type of acid and time between acidification and resin contact on the removal of radiostrontium from milk. J. Dairy Sci., 47, pp. 549-552.

Edmondson, L. F., Keefer, D. H., Douglas, F. W., Jr., Harris, J. Y. and Dodson, E. 1963. Comparison of the removal of radiostrontium from *in vivo* and *in vitro* labeled milk by ion exchange resins. J. Dairy Sci., 46, p. 1362.**

Allergens of Agricultural Products

Morris, R. S., Spies, J. R. and Coulson, E. J. 1964. Disc electrophoresis and gel diffusion of the castor bean allergenic protein, CB-65A. Federation Proceedings 23, March-April.

Spies, Joseph R., Coulson, Emery J. and Wells, Percy A. August 20, 1963. Deallergenization and detoxification of castor bean pomace. U. S. Patent 3,101,266.

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AREA NO. 3 - MEATS - PROCESSING AND PRODUCTS

Problem. Livestock production is the greatest single source of farm income: production of meat animals accounted for 30 percent of farm cash receipts in 1963, and the major part of U. S. farm land suitable for cultivation is used to produce feed for livestock. Hence any research which succeeds in stimulating an increase in the consumption of meat and other livestock products can provide a powerful stimulus to U. S. agriculture. For example, a one percent increase in meat consumption would require feed equivalent to about 80 million bushels of corn.

Increases in livestock consumption may be achieved through development of new or improved meat products, or through improved meat processing technology which results in lower costs. In addition, increases in the value of hides, animal fats and renderers' proteins will benefit the livestock industry by providing additional revenues which could permit reduction in meat prices (thus stimulating consumption) or which could flow back through the marketing channels in whole or in part to livestock growers and feed producers. For example, it is estimated that loss of the market for hides would cause an increase of meat prices that would result in a decrease of 2 percent in meat consumption. Such a decrease would eliminate a market for feed equivalent to 160 million bushels of corn. Conversely, an increase in hide values would operate in the opposite direction and would result in greater income to the livestock industry and in increased utilization of feed grains.

The attainment of an increase in livestock consumption requires both applied and basic research. Applied research is the forerunner of commercial practice and is an indispensable element in successful development. But applied research is based on the fundamental knowledge that is acquired through basic research, and represents the exploitation of this fundamental knowledge. The supply of fundamental knowledge must be maintained and expanded, if applied research is to be most effective and fruitful. The need for basic research has been pointed out by the Animal and Animal Products Research and Marketing Advisory Committee, by the National Agricultural Research Advisory Committee and by other responsible groups.

For reasons outlined above, research which succeeds in increasing meat consumption can have a powerful effect on American agriculture. The potential effect may be assessed from the facts that (1) meat has a high elasticity of demand (a 1 percent drop in retail meat prices will result in an 0.7 percent increase in consumption); (2) the production of 1 pound of livestock requires the equivalent of 7-8 pounds of feed grains; and (3) the U. S. per capita consumption of meat, 164 pounds in 1962, is only of the same order that it was 50 years ago and is below that of several other countries, including Australia (234 pounds per capita), New Zealand (222 pounds), Uruguay (234 pounds) and Argentina (166 pounds).

The attainment of increased meat consumption will require a vigorous and balanced program of research. There is a need for more applied research on processing and preservation, including time-temperature-tolerance studies on frozen meat, and on new and improved meat products. Of equal or greater importance is the need for more basic research on the physical, chemical and organoleptic characteristics of meat, and on the microbiology of meat.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving chemists, biochemists, and microbiologists engaged in both basic studies and the application of known principles to the solution of problems in the processing of meat and its products. The Department's research facilities are located at Beltsville, Maryland, and at Wyndmoor, Pennsylvania.

The Federal scientific effort devoted to research in this area totals 28.3 professional man-years. This effort is applied as follows:

(a) Research on microbiology of meat and meat products involves 8.0 p.m.y. at Beltsville, Maryland. In addition contract research at Iowa State University (1.6 p.m.y.) seeks to develop new cured products of distinctive flavors as the result of a study of fungi associated with meat processing.

(b) Product stability studies involve 4.5 p.m.y. at Beltsville. Contract research at Florida State University, concerned with meat tissue lipid oxidation and its prevention by antioxidants, was officially discontinued as of November 1963. A research grant (1.0 p.m.y.), recently executed at Florida State University, will be concerned with the relationship of heme pigments to oxidative rancidity in frozen meats. Under the P.L. 480 grant program, research is in progress at the University of Helsinki College of Agriculture, Helsinki, Finland, on the influence of fats on quality factors of dry sausage.

(c) Study of meat composition and quality involves 5.0 p.m.y. now at Wyndmoor, Pennsylvania, following transfer of the work from Beltsville, Maryland. A research contract (1.4 p.m.y.) at Louisiana State University involves investigations on selected characteristics of connective tissue and modification to improve meat tenderness. A research contract with Michigan State University (1.2 p.m.y.), recently executed, will be concerned with development of new smoked meat products. Research sponsored under P.L. 480 grants is under way at: (1) Low Temperature Research Station, Cambridge, England, on enzymes that attack the connective tissue of meat; (2) British Food Manufacturing Industries Research Association, Leatherhead, Surrey, England, on biochemical properties of pork muscle related to pigment formation during curing; (3) Politechnical University, Politechnika Gdanska, Gdansk 6, Poland, on antioxidant components of wood smoke used in meat curing.

(d) Meat flavor--its measurement and chemistry--involves 4.6 p.m.y. at Wyndmoor, Pennsylvania. A research contract (1.0 p.m.y.) has recently been negotiated at the University of Missouri to study the time-temperature-tolerance of frozen meats and the effect of freezing on meat flavor.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

State stations have an extensive program of meats research involving both basic and applied problems. Its scope extends from study of the effects of pre-slaughter treatment to development of new products.

Pre-slaughter factors considered are the effect of exercise and other stress, diet, environmental conditions, hormonal supplementation, and breed on quality, color, water binding capacity, structure and composition of meat. Physical and biochemical characteristics of the meat are determined and related to flavor and acceptability of the meat.

Among the basic chemical and biochemical studies is work on the mechanism of muscular contraction. Through the use of chemical analogs of adenosine triphosphate, the interaction of these analogs with actomyosin threads and with muscle fibers is observed under a number of different conditions of pH, temperature and ionic strength to see if important leads can be found as to the basic mechanism of contraction. In other fundamental studies, the free and bound water of muscle protein is determined and related to biochemical properties such as pH, buffering capacity, the acetic and basic groups, fat content and protein content of the muscle tissue. Gas chromatographic techniques are used to determine the fatty acid content and relative amounts and kinds of saturated and unsaturated fatty acids present in cooked and uncooked meat.

Previous research has demonstrated the presence of lysosomal enzymes in muscle tissue. Current work involves characterization of the complement of lysosomal enzymes and their relationship to tenderness. Purification and characterization of bovine muscle cathepsins is under investigation. There is much interest in the proteolytic changes of beef muscle during aging. Several studies of proteins involve the extractible proteins. In one study the muscle proteins are being extracted and separated and electrophoretic properties are being used to follow changes occurring in aging.

The chemical nature of the pigments in meat is under investigation. The color stability of freeze-dried meat and the time-temperature patterns of pigment fading in cured meat are examples of problems being researched. Other biochemical work is concerned with the carbonyl compounds produced during oxidation of fats and problems of rancidity development in pork fat. Continuing effort is devoted to identifying selected physical and chemical characteristics of fresh meat which may be related to quality or shelf life. Research in methodology centers around the search for means to objectively identify good quality meat. Histological studies are used to evaluate structural changes. Some work has been devoted to measurement of levels of blood enzymes and their evaluation as an index for prediction of beef quality.

As is the case with most foods, flavor research is undergoing increasing sophistication. Investigation of the components of flavor of fresh and cooked beef, lamb and pork are in progress. Chromatographic techniques are employed

and the role of fatty acids, volatiles and amino acids in flavor are being determined.

The influence of processing is being studied through determination of the reactions occurring during curing, smoking and aging of meats. Methods for quick aging are sought. The energy kinetics in freeze-drying; prefabrication problems; enzyme injection; and frozen storage problems are examples of other work in progress.

Microbiological programs deal with fresh, packaged and processed meat items. Bacteriological aspects of cookery, packaging, storage, curing, and self-service meats are being considered. Previous work has revealed radiation resistance in certain bacteria surviving ionizing radiation. This is now being extended to investigation of the metabolism and mechanism of radiation resistance in bacteria.

A portion of the meats research is conducted under the regional project, WM-33.

The total research effort on meat in 40 stations is approximately 64.3 p.m.y.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Microbiology of Meat and Meat Products

Meat, our most valuable agricultural product, is highly susceptible to microbial deterioration at all stages of its dressing and processing. Losses of meat due to spoilage total many millions of pounds annually in the United States. Such losses must inevitably result in increased prices to the consumer which in turn depress consumption and consequently decrease the demand for feed grains. Ordinary refrigeration does not control many of the microorganisms responsible for much of the spoilage. Microbial growth may be apparent at the surface due to propagation of bacteria, yeasts, or fungi. Microorganisms also indirectly bring about chemical changes by action of lipases and other enzymes synthesized by microorganisms. Basic research on lipolytic enzymes at EU represents a beginning in a very important phase of meat research.

Microorganisms not only cause much spoilage in meat but, selected and employed in the proper way, they result in products of improved flavor and/or desired preservation. Purposeful treatment of meat with the right types of microorganisms in a controlled manner is made use of in producing cured meats of improved flavor, general quality attributes and storage stability.

Effects of microorganisms on meat are by no means confined to refrigerator temperature and above. Meat quality is affected by microorganisms capable of growth near 0° C. Particular emphasis is being placed on the nutrition of these psychrophilic microorganisms and on their production of lipases. Attention has been directed during the past year to Pseudomonas fragi and its lipase including: (1) purification of the lipase preparation to 30-40 times its original activity; (2) study of the heterogeneity of the enzyme; (3) effect of the enzyme in decreasing the concentrations of carbonyls and peroxides in fresh and rancid lard emulsions. A preliminary study of Clostridium perfringens (capable of causing food poisoning) indicates that this organism can tolerate salts in concentrations employed in cured meats.

Flavor improvement of cured meats is being sought through a study of the interrelationship of temperature, curing substances, microbial metabolism, and microbial mutation. There is an increasing demand for quick-cured products of more distinctive flavor. Attempts are being made to develop mutations and control microbial action in order to enhance meat flavor.

In contract research at Iowa State University, fungi associated with meat processing are being studied in an effort to develop new cured products of distinctive flavors. Several meat curing plants were visited to obtain processing data and samples. These plants, each producing a type of product desired in its local market, employ a wide range of conditions in aging, smoking, and use of salts. This study seeks to answer questions concerning the desirable and undesirable changes caused by molds and yeasts in stored meats.

B. Product Stability Studies

These investigations are related to the chemical, physical and biological factors involved in development of oxidative rancidity in fat.

Changes resulting from oxidation of tissue lipids are characteristic of all situations where meat or meat products are stored for longer than a few days. Frozen, cured, dehydrated, and even canned products, as well as purified meat fats, all demonstrate these changes. At times these may result in desirable flavor development, but in most products they result in the unwanted changes we refer to as rancidity. A better understanding of the chemistry involved in the oxidation of meat fats will lead to better control of meat processing and storage and to improvements in many products.

The unsaturated fatty acid components of fats undergo multiple and complex reactions with oxygen that result in the formation of minute amounts of compounds containing carbonyl groups. Some of these carbonyls have a drastic effect on flavor at a concentration as low as a few parts per million. A number of methods are available in the literature for determining free carbonyl compounds. These methods are being compared and have yielded values that differ over a considerable range. The complexity of the mixture of carbonyls in oxidized fat necessitates development of an involved procedure to provide for identification and quantitative measurement of each compound. There is good reason to hope that the work in progress with the Girard T reagent will solve the problem of carbonyls determination. Work has been initiated on antioxidants that are effective and practicable for use in meat products. Investigation of oxidation inhibition will be extended to include antioxidants that occur naturally in meats.

Meat pigments and lipids can react under conditions in which the pigments act as oxygen donors and promote rancidity development. Cured lamb, for example, is an excellent product when freshly prepared but stores poorly even when frozen. Precooked frozen meats also suffer from a so-called "warmed over" flavor defect. Contract research on meat pigments and their relation to lipid oxidation at Florida State University was officially discontinued as of November 1963. Findings in this research, reported in the 1963 progress report, should be of great value in solving many practical problems in meat preservation.

A study is being carried on at the Institute of Meat Technology at the University of Helsinki by Dr. F. P. Niinivaara, under P. L. 480 grant, in which changes in the fat component of fermented sausages are receiving attention. Dr. Niinivaara is well known for his development of successful microbial inoculants for preparing fermented sausages of a sort not unlike the dry fermented sausages prepared in the United States. This project is a relatively new one, which succeeds a recently completed project on the changes in the nitrogenous components of fermented sausages during ripening. The present project is concerned with changes in the lipid constituents induced by the microorganisms used for inoculation. To date, the work has been concerned with the development of methods for the selection of suitable strains of

microorganisms and with the perfection of chemical methods for product analysis. The changes in the lipid constituents of the products under investigation are essential to flavor development. Future work of the project should help greatly in improving our knowledge of these changes and lead to new technological developments.

C. Meat Composition and Quality

Studies on composition and quality during the past year have been concerned with meat curing reactions, tenderness investigations, and influence of protein on tenderness and juiciness. Research on the composition of meat can be divided into two categories: (1) major components such as the proteins and fats and (2) constituents present in low concentration but which can have significant effects on properties such as flavor or water retention. Myosin comprises the larger fraction of fibrillar proteins in the live animal. After slaughter a large portion of the myosin polymerizes with actin to form actomyosin, which makes up a substantial portion of meat. Myosin also plays an important part in the glycolytic changes accompanying rigor. Physical-chemical reactions of myosin are important in developing a complete understanding of muscle structure and function. In the category of minor components, electrolytes are of great importance in controlling water retention in tissues. It has been previously determined in EU research that nearly all of the potassium, calcium and magnesium remain in the supernatant fraction of a sodium chloride extract of meat protein while a large portion of the zinc remains bound to the protein that is extracted by saline solution.

With transfer of the work on meat composition and quality and flavor investigations from Beltsville to Wyndmoor during the year, progress along these lines was hampered for a period. However, chemists have been recruited to fill a number of vacancies, necessary specialized equipment has been obtained, and research is now being accelerated.

Cured meats are produced in excess of 7 billion pounds annually in the United States, the principal products being ham, bacon, corned beef and sausage. This represents more than one-third of our pork usage and an important part of the beef production. The trend in cured meat processing is to reduce curing time and streamline methods to approach a continuous process. Commercial results have not been uniformly successful, and it is doubtful if further advancement can be expected from the empirical modification of old processes. Continued development of meat curing processes must await accumulation of new research results to point the way for a rational and scientific approach.

Three phases are receiving emphasis in the studies on meat curing reactions: (1) influence of zinc on the water-binding power of proteins; (2) reactions of myoglobin in fixing the color of cured meats; (3) certain problems in emulsion formation in processed meats. Beef fat is more difficult to use in emulsified meat products than is pork fat. Work is in progress to compare these properties of beef and pork fats that result in differences in emulsified processed meats.

Another study emphasizes the relationship of protein composition and distribution of meat tenderness and juiciness. It has been determined that fibrillar proteins are of great importance in meat tenderness. Beef myosin is being characterized.

A new line of research will be concerned with development of new and improved meat processing methods and products. First investigation to be considered will be related to methods for producing meat emulsions of superior quality and stability.

The purpose of a histochemical study, being conducted by contract at Louisiana State University, is to relate connective tissue composition with tenderness. The effects of nutritional stress and other treatments on the tenderness, structure and muscle biochemistry are being determined. The relationships will not be known until computer analysis is completed.

A P.L. 480-supported project on meat enzymology, under the direction of Dr. S. M. Partridge of the Low Temperature Research Station, Cambridge, England, is now nearing completion. The project is concerned with the effects of enzymes on muscle connective tissue. Most of the experimental work has dealt with the purification and chemical identification of certain specific entities of connective tissue and has actually resulted in the discovery of a hitherto unknown constituent of connective tissue. Pending final completion of the work, it is difficult to say what significance this substance, a mucoprotein, has for the meat tenderness problem.

An investigation of the chemical reducing system in pork muscle is being conducted by Dr. A. McM. Taylor of the British Food Manufacturing Industries Research Association, Leatherhead, Surrey, England, under P. L. 480 grant. It is a new project and succeeds an earlier completed one dealing with the chemistry of NO_2 reduction during meat curing. The earlier work showed that NO_2 is reduced to NO which reacts with pork myoglobin. The present work will attempt to discover just what it is in muscle tissue which could bring about the reduction. This research is particularly important, since the earlier project showed that only a fraction of the available myoglobin was actually converted to a nitroso pigment. An understanding of the chemistry involved will help the development of better curing methods.

A P. L. 480 grant study is being conducted by Dr. Tilgner of the Politechnical University of Gdansk, Poland, on the role of antioxidants in the smoking process. Dr. Tilgner has a considerable reputation for his work on smoke and meat curing and will, under this project, conduct research on the antioxidant properties of smoke, their chemical identification, and their quantitative relationships to smoke sources and smoking methods. Preliminary results reported by Dr. Tilgner showed that even very minute amounts of smoke have some antioxidant properties. Work is now in progress on identifying the antioxidants and in perfecting the analytical methods.

D. Meat Flavor -- Measurement and Chemistry

Further development of objective techniques for flavor and aroma evaluation of meats constitutes a much-needed area of research. In the past, research on the origin and development of undesirable flavors has been emphasized more than tracing down the naturally-occurring factors responsible for desirable flavors. Accentuation wherever possible of the positive factors is one of the requirements for extending the demand for meat.

In general, the objectionable flavors and odors develop after the animal is killed; they result from microbiological or chemical changes. An exception to this is the component of lamb flavor that many consumers dislike. Evidence has become available which indicates that meat from some lambs does not have this objectionable characteristic. Perhaps this results from a genetic factor, or it may be caused by a dietary constituent. Either of these possible causes may be controllable through further research.

Both the fat and lean portions of meat contribute to its flavor. It has been shown in previous EU research that pork, beef and mutton flavors arise from the respective fat fractions. The dried aqueous extracts of these meats in the fat-free state, upon pyrolysis, yield identical "aromagrams" in gas chromatography.

The physiological response to significant factors in flavor and aroma must eventually be correlated with the causative chemical compounds. One fraction obtained from an aqueous extract of beef gives the aroma of roast beef when heated. Preliminary experiments are in progress to identify the amino acid and carbohydrate components present in the aqueous extract and to relate eventually these precursors with development of cooked flavor.

The processing of smoked meats has traditionally been somewhat of an art, surrounded by a certain amount of secrecy. It is likely that the flavor and aroma of smoked meat products can be improved, given a more complete understanding of the smoking process. Preliminary experiments are being carried out to establish conditions for producing smoke, develop procedures for separating smoke fractions, and identify smoke components, and define conditions under which smoked meat of greater wholesomeness can be produced.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Microbiology of Meat and Meat Products

Alford, J. A., and Pierce, D. A. 1963. Production of lipase by Pseudomonas fragi in a synthetic medium. J. Bacteriol., 86, pp. 24-29.

Alford, J. A., Pierce, D. A., and Sulzbacher, W. L. 1963. Microbial lipases and their potential importance to the meat industry. AMIF Circ. No. 74, pp. 11-16.

Product Stability Studies

Gaddis, A. M., Ellis, Rex, and Currie, G. T. 1964. Carbonyls in oxidizing fat. VI. The Girard T reagent in the isolation and determination of micro amounts of n-aliphatic aldehydes and 2-alkanones. J. Food Science, 29,p.6.

Kwon, Tai-Wan, and Watts, Betty M. 1963. Determination of malonaldehyde by ultraviolet spectrophotometry. J. Food Science, 28, p. 628.

Sulzbacher, W. L., Gaddis, A. M., and Ellis, R. 1963. Oxidative rancidity in meat and meat products. Proc. of 15th AMIF Research Conference, Circ. No. 174, p. 111.

Meat Composition and Quality

Berman, Morris D., and Swift, Clifton E. 1964. Meat curing. The action of NaCl on meat electrolyte binding. J. Food Science, 29, p. 182.

Fryar, A. J., and Gibbs, R. J. 1963. Bovine muscle proteins. IV. Preparation of myosin and actomyosin. Experientia, 19, pp. 493-494.

Penny, I. F., Voyle, C. A., and Lawrie, R. A. 1963. A comparison of freeze-dried beef muscle of high or low ultimate pH. J. Science of Food and Agric., 8, pp. 535-543.

Scopes, R. K., and Lawrie, R. A. 1963. Port-mortem lability of skeletal muscle proteins. Nature, 197, pp. 1202-1203.

Swift, C. E., and Sulzbacher, W. L. 1963. Theory of emulsions. Physical and chemical makeup of sausage ingredients. Natl. Provisioner, 149, (18),12.

Voyle, C. A., and Lawrie, R. A. 1963. The demonstration of sarcoplasmic reticulum in bovine muscle. J. of the Royal Microscopical Society, 82, pp. 173-177.

Walters, C. L., and Taylor, A. McM. 1963. Biochemical properties of pork

muscle in relation to curing. Food Technology, 17, pp. 118-123.

Walters, C. L., and Taylor, A. McM. 1963. Differentiation of myoglobins and and hemoglobins. Nature, 198, pp. 996-997.

Walters, C. L., and Taylor, A. McM. 1964. Pig-heart myoglobin and its derivatives with nitric oxide. Biochim. Biophys. Acta, 82, pp. 420-422.

Walters, C. L., and Taylor, A. McM. 1964. The identification and estimation of nitric oxide by its absorption in alkaline sodium sulphite. Biochim. Biophys. Acta, 82, pp. 423-425.

Walters, C. L., and Taylor, A. McM. 1963. The utilization of nitrite by mitochondrial fractions of pork skeletal muscle. Proc. of 9th European Meeting of Meat Research Workers, Budapest.

Meat Flavor -- Measurement and Chemistry

Hornstein, Irwin, Crowe, P. F., and Sulzbacher, W. L. 1963. Flavour of beef and whale meat. Nature, 199, (4900), p. 1252.

AREA NO. 4 - ANIMAL FATS AND OILS--INDUSTRIAL UTILIZATION

Problem. The 4 billion-pound-per-year output of inedible fats is one of the major products of the livestock industry. It also is one of major concern, because while production of animal fats has more than doubled in the last 15 years, its principal outlet (in soap) has declined sharply, and is still declining.

The best answer to the question of what to do with huge amounts of fats is to find new uses through utilization research. Already utilization research has played a leading role in finding new uses for almost 1 billion pounds of animal fats, and thus helped retain markets for fats. Use of fat in animal feed which was developed through research, has now become number one use of inedible fats. There is need, however, for new uses not merely to retain or defend markets, but to expand them, and to upgrade the value of animal fats. The organic chemical industry presents a good opportunity for expanded markets, producing as it does a multitude of products--polymers, plasticizers, insecticides, herbicides, lubricants, paper chemicals--totaling 10 billion pounds. Animal fats possess "built-in" properties which make them potentially useful as raw materials to the chemical industry, but research must be done to realize this potential.

Both basic and applied research are needed; the basic to establish the fundamental facts as to composition, separation of constituents, preparation of chemical derivatives of constituents and determination of their physical and chemical properties; the applied to put these facts to work in developing new industrial products.

An increase of 1 cent per pound in the value of inedible animal fats would provide an additional revenue of $40 million to the livestock industry. This additional revenue will help the industry and growers in the same way as revenue from other animal products and by-products.

The attainment of an increase in the monetary returns from livestock requires both applied and basic research. Applied research is the forerunner of commercial practice and is an indispensable element in successful development. But applied research is based on the foundation of fundamental knowledge that is acquired through basic research, and represents the exploitation of this fundamental knowledge. The supply of fundamental knowledge must be maintained and expanded if applied research is to be most effective and fruitful. The need for basic research has been pointed out by the Commission on Increased Industrial Use of Agricultural Products, the National Agricultural Research Advisory Committee and by other responsible groups.

USDA AND COOPERATIVE PROGRAM

The Department has a broad program of basic and applied research at Wyndmoor, Pennsylvania, and at additional locations where contract and grant research is being carried out involving chemistry and physics, aimed at developing new and improved products from fats for use in industry. Total professional man-

years (p.m.y.) are 52.8. Of this, 14.1 p.m.y. are devoted to studies on chemical composition and the physical and chemical properties of animal fats at Wyndmoor. Studies involve fatty acid composition of animal fats using the latest advances in chromatography and other techniques; intra- and intermolecular structure of pure components and derivatives and factors that influence development of off-flavors in fatty foods. A research contract on the chemical and physical characteristics of organic peroxides involving 0.9 p.m.y. is going forward at the University of Pittsburgh, Pittsburgh, Pa. Research is underway at Villanova University, Villanova, Pa. with two contracts on spatial interrelationships within triglyceride molecules and on x-ray investigations of triglycerides and involves 0.7 p.m.y. each. Additional research sponsored by the Department under a P.L. 480 grant is now in progress at the University of Madrid, Spain, on the preparation of cocoa butter substitutes from animal fats.

Research on improved polymers, plastics, resins and lubricants involving 16.8 p.m.y. at Wyndmoor is conducted on the preparation of new products from fats through vinyl polymerization, condensation polymerization and synthesis of organic compounds for use as plastics, plasticizers and lubricants. A research contract with the University of Arizona at Tucson involving 0.3 p.m.y. deals with use of products derived from animal fats to synthesize plastics and other polymeric materials. In the current program an aspect of animal fat research is a contract with U. S. Industrial Chemical Company, New York on "Ethylene copolymerization with unsaturated fatty acids and gum naval stores." EU shares the effort to the extent of 0.4 p.m.y. in cooperation with SU. Additional research under way at the University of Aix-Marseille at Marseille, France with P.L. 480 funds concerns preparation of hydroxylated derivatives of animal fats for use in industrial products such as plastics and lubricants. 8.4 p.m.y. are being devoted at the Laboratory located in Wyndmoor to research on development of improved synthetic detergents based on animal fats, which includes preparation, testing of detergent power, and measurement of biodegradability of α-sulfo fatty acids and their esters, tallow alcohol sulfates and other fat derived materials; study of soap-detergent combinations for use as bar detergents is also included. A research contract with Lehigh University at Bethlehem, Pa. involving 0.5 p.m.y. deals with interfacial absorption characteristics of fatty acids. 9.4 p.m.y. are being devoted at Wyndmoor to exploratory investigations of new chemical derivatives of animal fats for use as chemical intermediates for industry. A research grant with the Hormel Institute of the University of Minnesota at Austin, Minnesota involving 0.6 p.m.y. provides for the investigation of the ozonization of animal fats.

PROGRAM OF STATE EXPERIMENT STATIONS

A limited program of work directed to the utilization of fats and oils is in progress at the State Stations. Work continues on the development of procedures for improved utilization of fats and is currently centered on separation of individual glycerides of natural fats by use of mercuric acetate addition. Research also involves development of methodology for, or production of, cis-trans isomerism to obtain a high yield without

polymerization. Other workers have developed a procedure for analyzing the triglyceride composition of natural fats by multiple chromatography. Under a study aimed at developing methods for the conversion of low grade fatty materials into industrially valuable products, a possible commercial method for producing elaidic acid from oleic acid has been developed.

Other research is directed toward identification of the components responsible for the flavors of auto-oxidized fats and the factors affecting their production. The use and role of antioxidants is also being investigated. Study of substrate specificity in lipases continues. The inter- and intramolecular specificity of lipases are being determined as a prelude to structural analysis of triglycerides. Another study deals with the effects of heating on the chemical changes occurring in commercial fats, including lard and combination animal-vegetable shortenings.

Utilization of fats in animal feeds as nutrients for energy production is another phase of this program. Studies involve relationships of chemical characteristics to digestibility, metabolism for energy production and storage in body tissues. Development of formulas, feeding trials and study of computer formulated least-cost feeds are related aspects.

A total of 2.7 professional man years is devoted to research on industrial utilization of animal fats and oils at the State agricultural experiment stations.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties; Autoxidation

1. Purification of lipids and determination of their structure. Knowledge of the composition and structure of animal fats is of primary importance to a better understanding of their behavior and to improved utilization in both food and non-food products. The development of newer analytical techniques, such as chromatography, has made it possible to separate many of the components of fats that was not possible by the older chemical methods. In carrying out this research a constant effort has been made to improve analytical techniques with particular reference to separation and analysis of fatty constituents.

After separation of complex natural products into their components, the intramolecular structure of each becomes the major problem. Further refinement in the newer techniques of infrared, NMR, and x-ray spectroscopy make achievement of these goals feasible. An important adjunct to use of these tools is the synthesis of model compounds of known chemical structure. All of these approaches are being followed.

Infrared spectra provide information from which the nature of the atomic bonds in a molecule can be determined and x-ray spectra, where adequate computer equipment is available, even permit the construction of a 3-dimensional model, providing that pure compounds of known chemical

composition are available for comparative studies; for example, pure oleic acid has the cis configuration around the double bond; elaidic acid, the positional isomer of oleic acid, has the trans configuration. In fatty acids with multiple double bonds these cis-trans relationships become more complex, since the number of isomers increases rapidly; e.g., with two double bonds four isomers are possible. Interpretation of spectra requires the availability of models of known structure. Synthetic reactions are available now by which such molecules can be constructed and significant progress is being made in this direction.

Another area of fat chemistry in which instrumental analysis is especially helpful is in determining the structure of addition products of unsaturated acids; for example, the structure of many compounds, formed by the addition of acetate, cyanoacetate, sulfur compounds and other agents to oleic acid has been elucidated.

In conventional techniques to determine the structure of organic compounds degradation of the molecule into smaller units is achieved through the action of chemical agents; for example, glycerides are hydrolyzed into fatty acids plus glycerol by the action of acid or alkali -- reagents which attack all three of the ester bonds at the same time, even though the middle ester bond is structurally different from the other two. Another tool with advantages for the structural chemist has resulted from progress in biochemistry; some lipases, enzymes which hydrolyze fats, are specific in that they only attack the two end ester groups in glycerides, leaving the middle one untouched.

Use of lipase, isolated from the pancreas gland of mammals, to hydrolyze fats has clarified the composition of some glycerides. Data obtained with pure synthetic triglycerides show the specific action of the pancreatic lipase for the 1,3 ester groups to be absolute and the technique to be usable as a criterion of purity for di- and triglycerides; the fatty acids of the monoglyceride fraction were all bound in the two position of the initial fatty triglyceride. Application of the procedure to lard showed that 83% of palmitic acid, but only 10% of stearic and 18 carbon unsaturated acids, occurred in the two position.

Further use of chromatographic methods has shown that it is possible to isolate the larger quantities of highly purified triglycerides which will be required in the study of the structure and physical properties of lard and other animal fats.

2. Autoxidation. A reaction characteristic of all fats containing unsaturation is called autoxidation; this is very important in all uses of fats, especially in food because some of the products of autoxidation are said to have toxic properties when present in significant amounts. Autoxidation reactions can be delayed by the addition of antioxidants, but some of these protective agents themselves are believed to form products with undesirable physiological effects under conditions conducive to fat oxidation. For these reasons elucidation of the mechanism of autoxidation

and discovery of means to minimize these changes in fats are extremely important to the livestock industry. The fact that intermediates in the oxidative process are both inherently unstable and highly reactive complicates the chemist's problem. Peroxides are a major intermediate; if they could be converted to stable products immediately upon formation, the analytical problem would be greatly simplified; so far this goal has not been reached.

In experiments on emulsified methyl oleate the rate of autoxidation under ultraviolet irradiation was more than 10 times as great as in the presence of added histidine and ferric ion without irradiation. Histidine and iron together increase the autoxidation rate about 8-fold over that in their absence, and result in a higher accumulation of hydroperoxides than with irradiation, in which more epoxides accumulate. Various methods of studying autoxidation of the doubly unsaturated methyl linoleate show reasonable agreement on the extent of oxidation as measured by gas liquid chromatography, peroxide value, percent conjugation and oxygen uptake.

The finding that rate of autoxidation of emulsified linoleate may be altered by varying pH, buffer or emulsifier demonstrates once again the complexity of emulsion oxidations and the danger of comparing the effect of a single variable in widely varying emulsion systems. The finding that ferric ion, normally a pro-oxidative catalyst, may act anti-oxidatively at certain pH's or with certain buffers and emulsifiers is surprising. This possibly may be explained by the fact that ionic iron can act to catalyze non-radical decompositions, thus diminishing the concentration of peroxy radical and curtailing the autocatalytic nature of the oxidation.

B. Improved Polymers, Plastics, Resins and Lubricants

1. Polymers, plastics and resins. Since the plastics and plasticizer fields offer larger potential outlets for chemical intermediates derived from animal fats, a wide variety of components and derivatives of animal fats are being investigated for use in these fields. Further improvements were made in quality of coatings obtained from the reaction of formaldehyde with hydroxyphenylstearic acid plus an epoxy cross-linking agent. These products are unaffected by solvents such as acetone and benzene and have resistance to 5% sodium hydroxide or boiling water. Vinyl esters of hydroxy acids copolymerize readily with vinyl chloride; however, the films are easily broken. Vinyl-9,10-dihydroxystearic acid yields products insoluble in the usual solvents.

Polymerization experiments with N-allylamides of fatty acids were completed. The products have excellent wax properties, better color stability than carnauba wax, and are even a potential economical replacement for it. Further search will be made for beneficial additives to silicone fluid through fat derived dichlorocyclopropane compounds.

In a P.L. 480 supported study of hydroxylated derivatives of fats at the Universite d'Aix-Marseille, Marseille, France, oleic acid was brominated

allylically with N-bromosuccinimide. Hydrolysis gave a mixture of unsaturated hydroxy acids which were separated by low temperature crystallization. Isomer distribution corresponded to a statistical allylic hydroxylation of the oleic chain.

Rate studies on the copolymerization of N-n-octadecyl acrylamide and acrylonitrile will be broadened to include a variety of solvents. N-n-alkylacrylamides and acrylonitrile polymerization with benzoyl peroxide initiator yield interesting reaction kinetics; the scope of this investigation will be enlarged. Selected N-n-alkylacrylamides will be made in substantial amounts for further studies on internal plasticizers. N-n-alkylacrylamide copolymers prepared up to now are too hard and brittle for conventional uses. Experiments will be run with an added third component designed to give better properties to the products. Lower cost N-allylamides for conversion to waxes will be explored through the direct preparation of mixed fatty acid amides from hydrogenated tallow.

Urethane foams containing the fatty acid derivative 9,10-dihydroxystearic acid as a starting material have been prepared. When ethylene oxide is the reagent used to introduce hydroxyl groups into the stearic acid derivative for later reaction with isocyanate groups to give the typical rigid structure of urethane foams, polymer formation takes place so fast that some problem arises in securing adequate mixing. These fat based products are equal in quality to some commercial products as measured by their density, compressive strength, percent open and closed cells, water absorption and resiliency. Better behavior during manufacture may be possible by use of propylene oxide in place of ethylene oxide as a source of hydroxy groups.

2. Lubricants. The introduction of phosphorus, by the addition of 2-ethylhexyl phosphonate to an allyl ester of dimer acid, into a fatty acid derived compound for lubricant tests gave a product equal to a commercial hypoid gear lubricant. Earlier wear tests on fatty esters substituted in the position next to the carboxyl group with an aliphatic long chain showed this type of compound to have promise as a lubricant or lube additive, particularly under conditions where the material will not be lost through volatilization.

C. Improved Synthetic Detergents

1. Soap-detergent combinations from inedible animal fats. Soap, at one time the major use for inedible animal fats, is being replaced more and more by synthetic products derived mostly from petroleum because of their more desirable detergent properties; however, their inherent chemical resistance to biochemical degradation has recently created sewage disposal problems so serious that legislation to control their use is a possibility. This situation has prompted extensive research to design more easily degraded compounds which still have the desired detergent properties and low cost features. Straight chain fat derived products are known which satisfy the technical requirements, and intensive research is underway to make these fat derived materials competitive with those derived from petroleum, for, after all, cost will decide which starting material will be used.

Whereas the major presently used synthetic detergents are characterized as branched chain alkylbenzenesulfonates (ABS), the proposed replacement based on petroleum is characterized as a linear ABS. By two tests, one simulating disposal in river water, the other in sewage treatment plants, neither type ABS receives a very high rating. Of 14 fat derived detergents which appear to have acceptable biodegradability and detergent properties 4 are believed to be economically feasible: sodium oleyl sulfate; tallow alcohol sulfates; and sodium salts of the isopropyl or butyl esters of α-sulfostearic acid. Of 26 compounds (α-sulfoesters of fatty acids and α-phosphono fatty acids) with potential usefulness in detergent preparations none showed any evidence of skin irritation by conventional pharmacological tests.

D. New Chemical Derivatives

1. Epoxidation. Polyunsaturated fats can theoretically be converted to the corresponding polyepoxides. Only with pure starting materials can one expect to get a separable mixture of products. A liquid isomer of 9,10,12,13-diepoxystearate has been isolated with an estimated purity of more than 90%. Hydration of the polyepoxide results in products that may contain a cyclic ether structure since they contain only one half the number of expected hydroxy groups per molecule. If polyhydroxy compounds of this type can be prepared economically they represent potentially useful ingredients for plastics. Epoxides are reactive molecules in the sense that they yield a variety of products depending upon reaction conditions; for example, boron trifluoride catalyzes their rearrangement to a ketone structure.

2. Properties and structural characteristics of organic peroxides. Fundamental studies on the crystal structure and basic physical properties of organic peroxides are being carried out through contract research at the University of Pennsylvania and at the University of Pittsburgh. Organic peroxides derived from animal fats are easily and inexpensively prepared, and they are labile compounds which undergo a wide variety of organic reactions. These studies will provide basic information on the structure of these peroxide derivatives and important thermodynamic data which will aid in the development of reactions for converting peroxides of fats and their derivatives to commercially useful chemicals.

The long chain dibasic peroxy acids, as was shown earlier for the monobasic series, also exhibit polymorphic behavior. A determination of the spatial configuration of crystalline organic peroxides will contribute not only to the development of new useful compounds, but also to our basic knowledge in this field.

Alpha hindered fatty esters with promising lubricant properties can be prepared by the reaction of a terminal olefin with a long chain saturated ester, using tert-butyl peroxide as a catalyst. Acceptable yields are obtained with methyl palmitate and monounsaturated hydrocarbons such as 1-decene and 1-dodecene.

The secret to many successful reactions with fatty acids is the peroxide group, a versatile and powerful reagent when properly controlled. Industry now uses peroxides to make a wide variety of organic compounds on a commercial scale; epoxy fatty acids are one example. Chemists have tended to avoid study of the basic molecular structure of peroxides partly because of the difficulty of preparing them in the pure state. More recent advances in instrumentation, especially x-ray analysis, and means of pinpointing atomic architecture are changing this picture. Polymorphism is a property which has been established in both mono and dibasic peroxy acids. In a contract with the University of Pennsylvania on peroxy acids and tert-butyl peresters measurement of their dielectric properties, kinetics of their decomposition and heats of combustion have permitted some conclusions about the molecular structure of the peroxy group. Aromatic peroxy acids are more powerful epoxidizing agents than aliphatic acids, and hence may be more useful in certain types of fatty acid oxidations.

3. Exploratory reactions. The synthesis from animal fats of phosphorus and nitrogen containing compounds designed to have industrial use in plastics, resins, detergents and lubricants continues. Dicarboxylic acids, widely used ingredients in the manufacture of polyesters and amides, have been prepared in the laboratory from fat derived saturated or unsaturated monocarboxylic acids by a number of reactions in acceptable yield as measured by the amount of crude product. Further research will show how successful these reactions can be when used for the manufacture of polymers where product properties and economics are also evaluated.

For free radical addition to the carbon-carbon double bond, methyl oleate and methyl undecylenate were selected for study. Addition of acetic acid gave a product which can be converted in good yield to the corresponding tridecanedioic acid; methyl oleate yields a 20-carbon dicarboxylic acid. Better yields of addition products are obtained with ethyl cyanoacetate; but conversion of this product to the desired polycarboxylic acid is satisfactory yield has not been attained. Enol esters, as vinyl stearate, are another group of widely used intermediates in the manufacture of polymers. Isopropenyl esters of some fat derived dicarboxylic acids have been made and tested for their reactivity with mono substituted diamides to yield polyimides, with diamines to yield nylon-like polymers with polyhydroxy compounds to yield polyesters. Laboratory success has been achieved. The addition of thiocyanogen to the double bond of methyl oleate yields a mixture of products the nature of which needs to be investigated before possible industrial use can be realized.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties: Autoxidation

Barford, R. A., Herb, S. F., Luddy, F. E., Magidman, P., and Riemenschneider, R. W. 1963. Alcoholysis of Vernonia anthelmintica seed oil and isolation of methyl epoxyoleate. J. Am. Oil Chem. Soc., 40, pp. 136-138.

Herb, S. F., Magidman, P., and Barford, R. A. 1964. A satisfactory GLC column for the determination of epoxyoleic acid in seed oils. J. Am. Oil Chem. Soc., 41, pp. 222-224.

Silvers, M. J., Turner, D. L., Rodalewicz, I., Giorando, N. (Jefferson Medical College Hospital) Herb, S. F., and Luddy, F. E. (EU). 1963. Evaluation of activity of phospholipids in blood coagulation in vitro. Thrombosis et Diathesis Haemorrhagica, 10, pp. 164-189.

Susi, H., Morris, S. G., Zell, T. E., and Scott, W. E. 1963. Internal hydrogen bonding, rotational isomerism and differentiation of 1,2 and 1,3-diglycerides by near infrared spectroscopy. J. Am. Oil Chem. Soc., 40, pp. 329-332.

Improved Polymers, Plastics, Resins and Lubricants

Jordan, Edmund F. Jr., and Wrigley, A. N. 1964. Monomer reactivity ratios of N-octadecyl acrylamide and N-allylstearamide. J. Appl. Polymer Sci., 8, pp. 527-532.

Maerker, G., Ault, W. C., and Port, W. S. 1963. Glycidyl esters. IV. Hydration of glycidyl stearate. J. Am. Oil Chem. Soc., 40, pp. 193-196.

Scholnick, F., Ault, W. C., and Port, W. S. 1963. Epoxy resins from fatty esters derived from cyclohexane and epoxycyclohexane. J. Am. Oil Chem. Soc., 40, pp. 229-231.

Shono, T., and Marvel, C. S. 1963. Preparation and polymerization of vinyl 12-hydroxystearate. J. Polymer Sci., A, 1, pp. 2067-2074.

Swern, D. and Sasin, R. August 20, 1963. Trialkyl 9(10)-phosphonostearates as plasticizers for vinyl chloride polymers. U. S. Patent 3,101,325.

Improved Synthetic Detergents

Haeberer, E. T., Maerker, G. 1963. Analysis of soaps with hydrogen bromide in glacial acetic acid. J. Am. Oil Chem. Soc., 40, pp. 274-275.

Maurer, E. W., Stirton, A. J., Ault, W. C., and Weil, J. K. 1964. Long-chain α-phosphono fatty acids, salts and esters. J. Am. Oil Chem. Soc., 41, pp. 205-208.

Weil, J. K., Smith, F. D., Stirton, A. J., and Bistline, R. G. Jr., 1963. Long-chain alkanesulfonates and 1-hydroxy-2-alkanesulfonates: Structure and property relations. J. Am. Chem. Soc., 40, pp. 538-541.

Maurer, E. W., Stirton, A. J., and Weil, J. K. May 10, 1964. Preparation of long chain alkylsulfuric acids and salts thereof. U. S. Patent 3,133,946.

Stirton, A. J., Bistline, R. G. Jr., Weil, J. K. and Ault, W. C. April 7, 1964. Salts of α-sulfonated fatty acid esters. U. S. Patent 3,128,294.

Wrigley, A. N., Smith, F. D. and Stirton, A. J. January 28, 1964. Oxyethylated diols, nitriles and soaps. U. S. Patent 3,119,848.

New Chemical Derivatives

Jeffrey, G. A., McMullan, R. K., and Sax, M. 1964. On the enantiomorphism of organic peroxy compounds in crystalline state. J. Am. Chem. Soc., 86, p. 949.

Kenney, H. E., Komanowsky, D., Cook, L. L., and Wrigley, A. N. 1964. Preparation and etherification reaction of fatty dichlorocyclopropanes. J. Am. Oil Chem. Soc., 41, pp. 82-85.

Rothman, E., Serota, S., and Swern, D. 1964. Enol esters. II. N-acylation of amides and imides. J. Organic Chem., 29, pp. 646-650.

Silbert, L. S., Siegel, E., and Swern, D. 1963. Peroxybenzoic acid. Organic Syntheses, 43, pp. 93-96.

Swern, D., and Silbert, L. S. 1963. Studies in the structure of organic peroxides. Anal. Chem., 35, pp. 880-885.

AREA NO. 5 - HIDES, SKINS AND LEATHER - PROCESSING AND PRODUCTS

Problem. To maintain the utilization of animal hides and skins at a profitable level there is need to find new products and processes to provide outlets for about 8 million cattlehides that are now available in excess of domestic needs. The foreign markets that currently absorb these surplus hides are also threatened by the increased hide production and decreased per capita use of leather(the principal outlet for hides)that have dislocated U. S. markets and caused prices to drop so precipitously in the last 10 years. To meet this problem there is need for upgrading the quality of raw hides and skins, for reducing the costs of producing leather, and for developing new and nonconventional products from collagen. To achieve these objectives research is needed to develop improved curing processes and agents, more effective control measures for(ante mortem)defects such as grubs, brands and parasite damage, and improved methods of take-off. Fundamental research is needed on the composition of hides to provide basic information on the chemical, physical and physical-chemical properties and reactions of collagen and other hide components for use in studies on chemical modification and on the development of new and improved products and processes. Development of new, more rapid and economic processes for curing, handling, unhairing and tanning hides is needed to reduce the cost of producing leather. There is also need for research on the chemical modification of hide proteins to develop leather products with such improved "built-in" properties as increased resistance to wear, scuffing and deterioration from perspiration, enhanced washability, drycleanability and improved dyeability.

USDA AND COOPERATIVE PROGRAM

The Department is conducting a broad program of basic and applied research on hides, skins and leather at Wyndmoor, Pennsylvania, and at additional locations where contract and grant research is being carried out; this involves chemists, biochemists, microbiologists and leather technologists.

The Federal scientific effort devoted to the overall program totals 27.2 professional man-years, as follows:

(a) Chemical and physical properties and structure of hides and collagen involve 7.3 p.m.y. at Wyndmoor. These investigations encompass exploratory research on cattlehide components and basic research on the chemistry of collagen. One line of investigations is concerned with the isolation and identification of cattlehide components, their chemical and physical properties, and their organization within the hide structure as related to leather properties. The second part of the program deals with the forces that control the stability and reactivity of collagen and the factors responsible for the unique physical properties of leather. Of special interest are the physical-chemical properties of collagen, its soluble components and its reaction products with modifying chemical agents. The University of Cincinnati, Cincinnati, Ohio, is conducting contract research on the noncollagenous

proteins of cattlehides involving 0.4 p.m.y.

Under a grant with Northwestern University, Evanston, Illinois 0.9 p.m.y. is being spent to study the physical properties of collagens.

In addition, research sponsored by the Department under the P. L. 480 program is in progress at: (1) University of Turku, Finland, on the fractionation of gelatin and soluble collagen; and (2) Central Leather Research Institute, Madras, India, on the reaction of polyphenolic tanning compounds with hide proteins (collagen) and hydrothermal shrinkage of collagen and leather.

(b) Chemical modification of hides involves 9.0 p.m.y. at Wyndmoor. This program is concerned with research on the reactivity of hide proteins with various organic and inorganic chemicals in the development of new products intended for specific uses.

In addition research sponsored by the Department under the P. L. 480 program is in progress at the British Leather Manufacturers Association, Surrey, Great Britain for the study of chemically reactive compounds to improve leather stability.

(c) New and improved processing involves 8.0 p.m.y. at Wyndmoor. Research is aimed at developing better methods for unhairing animal hides and skin, for imparting deterioration resistance to leathers, and for processing hides into leathers possessing special properties. The University of Cincinnati, Cincinnati, Ohio, is conducting research on the abnormalities of leather characterized by a depleted mush texture involving 1.4 p.m.y.

Additional research under P. L. 480 grants is in progress at: (1) British Leather Manufacturers Association, Surrey, Great Britain, for a study of deterioration of leather by sweat, chemicals and heat; (2) Experiment Station for Leather Products, Naples, Italy, for research on the cause of "red heat" and on improved tanning methods for United States hides; (3) Central Leather Research Institute, Madras, India, on the interrelation of hide quality with the rate of tanning and efficiency of tanning; and (4) Leather Research Institute, T.N.O., Waalqijk, Holland,on kinetics of chrome tanning.

(d) Utilization of animal protein residues involves research in cooperation with the National Renderers Association, who support one Senior Fellow and one Junior Fellow at Wyndmoor, Pa., to conduct basic studies on the evaluation of meat and bone meal fractions of residues from fat rendering operations. The Department of Agriculture participates in this program to the extent of supplying supervisory and research leadership amounting to 0.2 p.m.y.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

State stations reported no research in this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical and Physical Properties and Structure of Hides and Collagen

Basic research on the molecular architecture of collagen, the major structural protein of vertebrates and amounting to about one-third of total body protein, is increasing. For instance, a meeting was held at Western Reserve University in February, 1964, just to discuss the cross-linkages in collagen. It is apparent from the proceedings that considerable progress is being made in cataloging probable cross-links and in developing new research techniques. This should result in a more rapid accumulation of information in the future. It is also apparent, however, that only a start has been made in describing the structural differences in collagens from different sources and in understanding the role of various cross-links.

1. Chemical and physical properties of collagen. Research in the utilization area has included comparative studies on the fiber structure of collagen from untreated hides, hides unhaired with lime or enzymes, and chrome-tanned leather, using both visual and electron microscopy. The abrupt change in size of collagen bundles from the surface to the center of the skin may have significance in the effects of processing on leather properties; gross structure, as revealed by visual microscopy, shows greater differences than submicroscopic structures as revealed by electron microscopy.

These fundamental studies on the structural features in hides and their correlation with properties of leather should yield information of use in developing products with improved properties. Studies on dispersion of insoluble collagen show that care must be exercised in the swelling step to prevent excessive degradation. The weakness of reconstituted films seems to correlate with degree of fiber damage as observed in the electron microscope. Reconstitution of hide collagen into other products has considerable potential as an outlet for the poorer areas of hides that are now being discarded or made into nonprofitable leather items. The torsion test gave satisfactory measurement of stiffness of sole leathers.

Investigations on the mode of reaction of polyphenolic compounds with collagen are being supported by a P. L. 480 grant at the Central Leather Research Institute, Madras, India. When collagen samples are swollen the rate of fixation of tannins was found to decrease in the following order: Quebracho, wattle, chestnut, myrabolams; this fits the general experience in sole and upholstery leather tanning. The grantee concludes that tanning is mainly by hydrogen bonding of polypeptide and amide groups with amino groups playing only a small role.

The P. L. 480 grantee at the University of Turku, Finland, has reported further on the specific properties of collagen and gelatin that are applicable to the development of expanded uses of animal hides and skins. The use of starch gel electrophoresis has made possible the separation of gelatinized

collagen into at least six different fractions. The remnants of these collagen subunits were also recognized in samples of commercial gelatins. Cross-linking in collagen with advancing age was demonstrated by stress-strain studies on the insoluble fraction of rat skin collagen and by the α- and β-subunits demonstrated by electrophoretic analysis of heat denatured extracts.

2. Effect of electrolytes and lipid components on hide properties. Dialysis of soluble collagen indicates the presence of a wide range of amino acids and small peptides. These constituents may influence the measurement of representative values of the physical properties of collagen. Total lipids were determined in each of five stratigraphic layers of hide; in general, the higher content of lipids occurs in the layer adjacent to the flesh with similar amounts in the center region. It is recognized that domestic feeder cattlehides contain greater quantities of fat throughout their structure which creates problems in tanning and processing. Information on the nature and distribution of fats in hides may enable tanners to correct some natural hide defects. Fundamental information resulting from this research should lead to a better understanding of the structural composition of animal hides.

Since the continued profitable utilization of animal hides will depend to some extent upon the development of new products from collagen, information on its behavior toward dispersing agents and to changes in influence of ions will aid in the development of a process for the dispersion and reconstitution of collagen and may lead to new items containing its characteristic properties.

3. Noncollagenous proteins of cattlehide. In contract work at the University of Cincinnati it has been shown that extraction of hides with brines containing 5 to 10% salt at room temperature gives the maximum yield of extractable protein. Brine curing of hides has been increasing rapidly in the last decade, and there are many conflicting reports in the trade about the leather making qualities of hides cured in this manner. Therefore it is important to study these soluble proteins to find out if their effect on quality is deleterious and if so how this may be avoided.

B. Chemical Modification

1. Improved water repellency for leather. Leather will have increasing competition from synthetic materials. Any improvements that can be made economically are highly desirable. One possibility is improvement in water repellency. Previous reports have outlined progress in this area. Further research on chrome tanned hides retanned with glutaraldehyde has led to excellent water repellency properties at lower cost. The process essentially consists of treating the tanned hide with an alkenyl succinic acid, followed by exposure to the water repellent such as Scotchguard, Quilon, or a silicone.

2. Chemical treatments. Some commercially available cyclic urea derivatives such as urons and triazones have shown promising tanning ability at alkaline pH; further work will be needed to find optimum conditions and to indicate the type of reactions involved. Other chemical reagents which may either produce a tanning effect or introduce new functional groups into hides which in turn

will react with tanning agents are being tested.

C. New and Improved Processing

1. Enzymatic unhairing of hides and skins. Extensive efforts to replace the lime unhairing process in leather manufacture with enzyme unhairing have been only partially successful. Evidently liming performs a specific and important, although little understood function in leather making. If the enzyme process could be applied to a substantial part of leather production, the waste disposal problems of lime unhairing would be eliminated. Research on tanning enzyme unhaired hides into good shoe upper leathers will continue with experiments on combination tannages and processing adjustments.

2. Improving the deterioration resistance of leather. For the past five years the British Leather Manufacturers Research Association in Surrey, England, has conducted research under a P. L. 480 grant to obtain information on the mechanism by which leather deteriorates under the action of moist heat, chemicals, and perspiration. The completed work clearly shows that moist heat is one of the chief causes of the deterioration of leather, the damage being due to degradation of the skin protein collagen and also to changes in the tanning agent. In wear, damage is essentially due to moist heat, mitigated or accelerated by the constituents of perspiration. The effect of such factors as the nature of tanning agent, pH and moisture content on the breakdown has been clarified and the importance of cross-links in maintaining structure of the protein has been emphasized. These studies explain why glutaraldehyde tannage (a USDA development) imparts to leathers a degree of resistance to deterioration.

3. New tanning processes. Further improvement in the leather manufacturing process depends upon the discovery of new economical treatments which will produce better or new properties in the product. Research with dialdehyde starch and glutaraldehyde tannage has made further progress. Until the price of dialdehyde starch declines to a level predicted to be possible, its wide scale use by the leather industry is improvavle. Glutaraldehyde is already in widespread us for production of leathers with increased resistance to deterioration from perspiration.

With P. L. 480 support the Central Leather Research Institute, Madras, India, is studying the relation of hide quality to tanning rate. Under conditions in India the simple commercial chrome tanning process gives as good a product as any of five other tested processes. Rate of chrome tanning and fixation varied in different areas of the hide because of the variable structure. Lime splitting of the hide did not adversely affect the physical properties of the finished leather.

4. Investigations on "red heat." The Stazione Sperimentale per l'Industria delle Pelli e delle Materie Concianti of Naples, Italy, has a P. L. 480 grant to study the causes and prevention of "red heat" damage that occurs in salted hides. A laboratory survey of chemical additives to salt reveated several effective germicides. Four of the most promising were tested in curing tests

completed at the Omaha plant of Armour and Company. The hides cured with the treated salt were shipped to Italy, where the grantee will evaluate the efficacy of the treatments as preservatives for hides during curing and transoceanic shipment. Work on the physiological and cultural characteristics of the microorganisms associated with "red heat" has been completed and reported in five publications.

5. Conversion of U. S. cattlehides into sole leather in Italian tanneries. Work is approaching completion under a P.L. 480 grant at the Stazione Sperimentale per l'Industria delle Pelli e delle Materie Concianti, Naples, Italy, on the development of processes for converting American packer hides into sole leather at Italian tanneries. The process developed in the laboratory was tested in five cooperating tanneries. Three tanneries that adopted the suggested procedure without modification obtained clean and technically suitable leathers. This conclusion was supported by data on chemical and physical analysis. Shoes were also soled with these leathers, and comparative wear tests are in progress.

D. Utilization of Animal Protein Residues

Investigations supported by the National Renderers Association are concerned with evaluating the quality of meat and bone meals from fat rendering residues by characterization of the proteins present. Over 2 billion pounds of these by-products of the rendering industry are available annually. Analyses of various fractions prepared from representative meals give a better picture of the nutritional value of the main protein fraction. Collagen, the major protein component, is deficient in some essential amino acids; principally tryptophane and methionine. It is technically feasible to fortify these animal protein residues with certain amino acids to give them better nutritional balance; for example, methionine is commercially available at a reasonable price. If this were done, it would promote uniformity of composition and the improved quality would potentially enhance the selling price of the meal.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical and Physical Properties and Structure of Hides and Collagen

Bitcover, E. H., Gruber, H. A., and Mellon, E. F. 1964. The stable linkages between epoxy resins and collagen. J. Amer. Leather Chem. Assoc., 59, pp. 15-22.

Botan, E. A., and Chouinard, A. E. 1963. A histochemical study of the basement membrane in cowhide. J. Histochem. and Cytochem., 11, pp. 482-484.

Grosskreutz, J. C. 1964. The fibrillar structure of collagen from enzyme and lime unhaired hides. J. Amer. Leather Chem. Assoc., 59, pp. 126-135.

Happich, W. F., and Eddy, C. R. 1963. Determination of the triazine ring in melamine-tanned leather by infrared analysis. J. Amer. Leather Chem. Assoc., 58, pp. 271-278.

Kahn, L. D., Calhoun, R. R., Jr., and Witnauer, L. P. 1963. Modification of the Brice light-scattering photometer for use in photoelectric polarimetry by the Rouy method. J. Appl. Polymer Sci., 8, pp. 439-445.

Khanna, J. K., Nancy, S. C., and Nayudamma, Y. 1964. Effect of locational variation in the hide on processing and properties of chrome-tanned upper leather. Symposium on Recent Advances in Mineral Tannages (Central Leather Research Institute, Madras, India), pp. 19-28.*

Kulonen, E., Virtanen, U. K., and Salmenpera, A. 1962. Fractionation of gelatins with carboxymethyl cellulose. Acta Chem. Scand., 16, pp. 1579-1585.*

Mellon, E. F., Audsley, M. C., and Gruber, H. A. 1963. Evaluation of commercial photographic developers which intensify silver halide spots on paper chromatograms. Anal. Chem., 35, p. 1326.

Mellon, E. F., Audsley, M. C., Viola, S. J., and Naghski, J. 1963. The contribution of hair follicles and sebaceous glands to the void space of hides. J. Amer. Leather Chem. Assoc., 58, p. 514.

Nikkari, T., and Kulonen, E. 1962. Studies in experimental lathyrism. I. Non-collagen components of tissues. Biochemical Pharmacology, 11, pp. 923-929.*

Nikkari, T., and Kulonen, E. 1962. Studies in experimental lathyrism. II. On the properties of collagen. Biochemical Pharmacology, 11, pp. 931-936.*

Viljanto, J., Isomaki, H., and Kulonen, E. 1962. Effect of aminoacetonitrile, iproniazid and semicarbazide on the tensile strength of experimental granulation tissue. Acta Pharm. Scand. Edita, 19, pp. 191-198.*

* Work done under a P.L. 480 foreign research grant.

Viljanto, J., Isomaki, H., and Kulonen, E. 1962. Effect of an anabolic steroid on the tensile strength of granulation tissue in various nutritional states. Acta Endocrin., 41, pp. 395-399.*

Viljanto, J., and Kulonen, E. 1962. Correlation of tensile strength and chemical composition in experimental granuloma. Acta Path. et Microbiol. Scand., 56, pp. 120-126.*

Viljanto, J., Majaniemi, T., and Kulonen, E. 1962. Observations on the thermally induced isometric tension in collagenous fibers of rat tail tendon with reference to lathyrism. J. Amer. Leather Chem. Assoc., 57, pp. 525-540.*

Weaver, E. A. 1963. The determination of phosphorous in different leathers by oxygen-flask combustion. Analyst, 88, p. 736.

Weaver, E. A. 1964. Devices useful for oxygen-filled flask combustions. Chemist-Analyst, 53, pp. 18-19.

Chemical Modification

Bowes, J. H. 1964. Factors in the stability of vegetable-chrome combination-tanned leathers. II. The extraction of chromium from chrome leather by vegetable tanning extracts. J. Amer. Leather Chem. Assoc., 59, pp. 190-200*

Bowes, J. H., Moss, J. A., and Young, F. S. 1964. A study of the changes in the ionic and complex-bound sulfate of chrome leather by radioisotope techniques. J. Amer. Leather Chem. Assoc., 59, pp. 136-147.*

Bowes, J. H., Moss, J. A., and Young, F. S. 1964. Factors in the stability of vegetable-chrome combination-tanned leathers. I. Interactions of the constituents of vegetable tanning extracts with chromium. J. Amer. Leather Chem. Assoc,, 59, pp. 174-189.*

Bowes, J. H., and Raistrick, A. S. 1963. The action of heat and moisture on leather. IV. The effect of chrome content and detannage by sodium lactate on the resistance of chrome leather to moist heat. J. Amer. Leather Chem. Assoc., 58, pp. 190-201.*

Bowes, J. H., and Raistrick, A. S. 1964. The action of heat and moisture on leather. V. Chemical changes on collagen and tanned collagen. J. Amer. Leather Chem. Assoc., 59, pp. 201-215.*

Cater, C. W. 1963. The evaluation of aldehydes and other di-functional compounds as cross-linking agents for collagen. J. Soc. Leather Trades' Chemists, 47, pp. 259-271.*

* Work done under a P.L. 480 foreign research grant.

New and Improved Processing

Filachione, E. M., Fein, M. L., and Harris, E. H., Jr. 1964. Tanning with glutaraldehyde. The Leather Manufacturer, Feb. 1964.

Formisano, M., and Meduri, A. 1963. Ricerche Sul 'calore rosso' delle pelli salati. V. Su di un caso di depigmentazione degli alofili cromogeni precedemente isolati e descritti. Bollettino della Stazione Sperimentale per l'Industria della Pelli e della Materie Concienti, 39, No. 4.*

Formisano, M., Simoncini, A., and Meduri, A. 1963. Richerche Sul 'calore rosso' delle pelli Salate. IV. Studio dei mezzi per prevenire l'alterazione. Bolletino della Stazione Sperimentale per l'Industria della Materie Concienti, 39, No. 1.*

Happich, W. F., and Windus, W. 1963. Perspiration resistance of leather tanned with tetrakis(hydroxymethyl)phosphonium chloride - resorcinol. J. Amer. Leather Chem. Assoc., 58, pp. 646-653.

Roddy, W. T., Gilsdorf, J. G., and Cordon, T. C. 1963. Studies of evaluation of an enzyme process for unhairing cattlehides. J. Amer. Leather Chem. Assoc., 58, pp. 624-637.

Wiegand, C. J. W., Morriss, F. V., and Windus, W. 1963. Chemical comparison of hides after enzyme unhairing and lime unhairing. J. Amer. Leather Chem. Assoc., 58, pp. 568-576.

Windus, W., and Happich, W. F. 1963. A new tannage - tetrakis(hydroxymethyl) phosphonium chloride - resorcinol. J. Amer. Leather Chem. Assoc., 58, pp. 638-645.

Windus, W. 1963. Practical tanning with glutaraldehyde. J. Soc. Leather Trades' Chem., 47, pp. 524-534.

* Work done under a P. L. 480 foreign research grant.

AREA 6. POTATOES - PROCESSING AND PRODUCTS

Problem. The potato industry, faced with a continuing decline in the consumption of fresh potatoes, is becoming more and more dependent upon the development of new and improved processed products to maintain markets and to avoid recurring economic disasters. Crop perishability, supply fluctuations, and the inelasticity of demand, result in wide swings in price with even slight surpluses. In producing areas having a substantial processing industry, depressive lows are moderated by advance contracting by processors prior to harvest. However, in many important potato growing areas processing has not yet developed, and vulnerability not only still exists, but is exaggerated by the growing competition of processed potato and other competing food products. A continuing improvement in processed potato products is clearly required if processing is to expand fast enough to offset the progressive decline in use of fresh potatoes.

Lack of adequate knowledge concerning the chemical constituents, physical properties, and enzyme systems in potatoes is limiting development of new and improved processed products and processing methods. Basic research on composition is needed to provide fundamental information on which an applied research program can be systematically and effectively built. Recently-developed techniques make it possible to isolate and characterize the constituents responsible for flavor, color, odor, and texture of many processed food products. Application of these techniques to potatoes and potato products should make it possible to improve the quality of present products, both freshly processed and following storage, and provide a basis for technological and engineering studies in new product development.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program of basic and applied chemical and engineering research on studies related to processing. The work of the EURDD, involving the services of chemists, biochemists, food technologists and chemical engineers at Wyndmoor, Pennsylvania, is conducted in cooperation with the Maine Agricultural Experiment Station and several other stations, which supply potatoes of known cultural history. The chemical research program includes: evaluation of the effects of variety, location of production, storage conditions, and tuber solids content on potato composition with particular respect to nitrogenous constituents; principal acids and other factors related to discolorations such as after-cooking discoloration; preliminary studies on the lipids, which are believed to occupy an important role in storage stability of processed potato products, particularly dehydrated foodstuffs. The Eastern Division's engineering and development research program seeks to improve the quality, nutritive value and storage stability of dehydrated potato products and to develop more convenient types of dehydrated products, such as "instantized" pieces that cook quickly. The Red River Valley Potato Processing Laboratory, East Grand Forks, Minnesota, has been established to conduct investigations

relating variety and other raw material characteristics to quality of established forms of processed potatoes. This new Laboratory is operated jointly by the Red River Valley Potato Growers Association, University of Minnesota, North Dakota State University and the Agricultural Research Service with the Engineer-in-Charge reporting to Wyndmoor.

The Federal (EU) scientific effort devoted to this area totals 14.4 professional man-years. Of this total, research on chemical composition as related to processing characteristics comprises 8.5 p.m.y. and research on dehydrated potato products amounts to 2.5 p.m.y. Research on new and improved processing technology amounts to 3.4 p.m.y., including 1.0 p.m.y. for the Red River Valley Processing Laboratory.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

For many years State stations have conducted research on problems related to potato utilization. Studies designed to evaluate, by biochemical and physical means, the effects of such production variables as fertilization, variety, location of production, harvest maturity, handling and storage on composition and use of potatoes are continuing.

The trend toward increased processing has added emphasis to work designed to evaluate processing quality of potatoes. A prime problem associated with the rapid growth of potato processing is maintaining quality of tubers during extended storage. Production variables and storage conditions affect potato composition. Mechanical harvesting systems frequently affect potato quality and composition. Yields; total solids; suitability for french frying, chipping, boiling, mashing and baking; and sugar content are routinely determined. More thorough chemical composition studies are carried out to determine content of phenolic acids, nitrogen, nitrogen-free organic acids, amino acids and reducing sugars as a means for better understanding utilization problems.

Interest in problems of discoloration and the role of nitrogenous compounds in enzymatic blackening leads to studies on the color reactions of polyphenol oxidase plus chlorogenic acid and/or caffeic acid with amino acids found in potatoes. Recently work is being initiated to determine the relationship of lipid content, both total and fatty acid composition, to discoloration of potatoes. The relationship of chemical and physical properties of potatoes to sloughing is also being studied.

New techniques for conduct of flavor investigations are applied to potatoes and potato products. For example, gas chromatographic techniques are used to study the volatile compounds of potato chips as a part of the attempt to isolate and identify the chemical compounds responsible for the flavor and aroma of chips. This work is tied in with efforts designed to find ways to maintain or extend fresh potato flavor and improve chipping quality or shelf-life.

Product research includes work designed to determine physical factors affecting the color or lack of brightness of reconstituted dehydrated potatoes. The relationship of specific gravity and starch content to the texture of frozen french fried potatoes is being investigated. Another study deals with the effect of pre-preparation, preparation, and post-preparation techniques on the quality, yield and cost of potatoes prepared in quantity.

The potato utilization research program also includes investigations designed to work out new or improved uses for sweet potatoes. New methods of processing grades currently not acceptable by the fresh market are sought. Suitability for canning, freezing and dehydration is determined. Means of using sweet potatoes in new products or other food products are investigated.

The total number of professional man-years devoted to potato utilization research is 7.7.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition as Related to Processing

1. Nitrogenous constituents. Individual extractable amino acid analyses of 1962 crop samples of Katahdin, Russet Burbank, Kennebec, Red Pontiac and Cobbler grown in Maine, New York, Pennsylvania, Red River Valley area, Wisconsin and Idaho were so different from corresponding data from the 1961 crop that the 1963 crop is being included to obtain a range of values considered representative. Analyses have been completed for about one-third of the samples of the 1963 crop.

Statistical analyses of the 1959 and 1960 storage studies have been completed. With the exception of glutamine and asparagine, the twenty-odd free amino acids in potatoes change very little during nine months of storage. On a fresh potato basis the data indicates that regardless of the total solids content, the potato stores the same amount of nitrogen and amino acids.

2. Basic studies on the after-cooking discoloration pigment. Potato protein can readily take up about 10 times as much iron as naturally present in the potato. Protein extracted from the stem end of the tuber, where after-cooking discoloration is more intense, is capable of complexing more iron than protein from the bud end. This correlates with analysis of discolored potatoes where stem-end protein contains 2 to 5 times as much iron as bud end protein and the degree of blackening is greater at the stem end. Of a number of amino acids tried in a model system in place of potato protein, only arginine and gamma-aminobutyric acid produced a black product.

3. Basic studies on potato lipids. Efforts were directed to improving techniques for extracting the lipids from the potato. Freeze drying of samples of whole potatoes prior to lipid extraction is underway in an attempt to minimize presence of artifacts in the extract.

B. Dehydrated Potato Products

1. "Instantized" pieces. Explosive puffing has been successfully applied to french fry strips and to thin slices of potato. Thin slices are now manufactured by several companies and widely used for salad, home style fries, au-gratin and scalloped potatoes. "Instantizing" is expected to cut the time of home preparation of such foods to about one-fourth that currently required.

2. Flakelet product. Blending 20% by weight of dry skim milk with dried flakelets makes it possible to use boiling (instead of tempered) water in reconstitution of the dry mix to a mashed potato product.

C. New and Improved Processing Technology

1. Texture and color of french fries. Physical tests and chemical analyses on raw potatoes and on corresponding french-fried samples were determined for four varieties of potatoes from the Red River Valley. Horticultural Crops Branch, AMS, is collecting data on sub-samples of the four varieties. Frying temperature and other processing variables are being investigated.

2. New Laboratory. The Red River Valley Potato Processing Laboratory was occupied by the cooperators as of March 24, 1964. Arrangements have been made with the Red River Valley Potato Growers Research Farm to plant required acreage of specified varies desired for experimental work.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition as Related to Processing

Fitzpatrick, Thomas J., Talley, Eugene A., Porter, William L. and Murphy, Hugh, J. 1964. Chemical composition of potatoes. III. Relationships between specific gravity and the nitrogenous constituents. American Potato Journal, 41, pp. 75-81.

Heisler, E. G., Siciliano, James, Treadway, R. H., and Woodward, C. F. 1963. After-cooking discoloration of potatoes. Iron content in relation to blackening tendency of tissue. J. Food Science, 28, pp. 453-459.

Dehydrated Potato Products

Cording, J., Jr., Sullivan, J. F., and Eskew, R. K. 1964. Puffing gun process produces quick-cooking dehydrated potato pieces. Food Engineering, 36, (6) pp. 49-52.

AREA NO. 7. VEGETABLES - PROCESSING AND PRODUCTS

Problem. Vegetable growing occupies over 3 million acres, with a yearly farm value of a billion dollars. Utilization as processed rather than fresh vegetables provides a constant source of supply with less price fluctuation. Basic compositional research is needed to provide knowledge of the constituents responsible for color, flavor and texture of vegetables and the changes these constituents undergo during processing, storage, and distribution. There is also need for application of these results to developmental research on new products and new and improved processing technology. Consumer preference is shifting to "convenience" foods. An even greater emphasis on quickly prepared foods is evident in modern military feeding where high bulk density, nonrefrigerated, and rapidly rehydrating products are of primary importance.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program employing chemists and chemical engineers in basic and applied research on vegetable processing and products. The Federal work is conducted at Wyndmoor, Pennsylvania. The scientific effort assigned to this area totals 4.5 professional man-years and is currently engaged in research on new and improved products and processing technology.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

State stations have a continuing long-term research effort devoted to vegetable processing and products. Research on the adaptability and evaluation of vegetable varieties for processing is a standard service to plant breeding programs. Before introduction of new varieties, processing yields and processing characteristics are determined. This type of research extends to consideration of the effects of various production variables on processed product quality. Effects of maturity at harvest, mechanical harvesting, fresh product characteristics, post-harvesting handling and storage are examples of problems under study. The degree of correlation or association between color and flavor in fresh and in processed items is always of major concern.

Chemical composition and physical properties are also related to product acceptance and quality. This research ranges from standard composition studies to basic studies of special components. For example, the noncellulosic constituents of plant cell walls are being investigated. The nitrogen compounds in mushrooms are determined and evaluated as a function of previous history of growth, handling and processing of mushrooms. A continuing analysis of the biochemical changes that occur in vegetables at the different stages of maturity is made. Other studies deal with determination of the antioxidant properties of chile and the antioxidant effects exerted in the various kinds of meats. Estimates of the metal complexes of chlorophyll derivatives in processed foods and their effect on the color of processed foods are made

through processing experiments conducted under controlled conditions of metal contamination in the laboratory or in commercial processing plants. The role of enzymes in chemical and physical changes in processed foods is studied through use of purified enzyme systems, substrates and reaction products.

In order to obtain a better understanding of the reasons for changes in flavors during processing and storage and for the development of off-flavors, a comprehensive program on flavors in processed foods is in progress. Heat-induced flavors; lipids in flavor, bitter flavor of carrots, and natural fresh flavors of vegetables are all under study.

Microbiological research extends from study of the natural flora found on fresh vegetables to studies of contaminants found in commercially processed foods. Methods for microbiological examination of foods are being developed. Physiological, morphological, and nutritional variation among important organisms are determined to facilitate control of the organism or essential understanding of the role of the organism in desirable or useful applications. Bacterial endospores receive much study. It is hoped that this work will lead to the improvement of present methods of sterilization and food preservation. The radioresistance of bacterial endospores and use of combined antibiotics and heat are carefully researched to provide information of use in developing new and improved procedures for canning vegetables. Food poisoning organisms are the object of continuing interest. The incidence of spoilage organisms, survival patterns, and means of control are being investigated. Studies on the effect of carbon dioxide inhibition of microbial growth are in progress. The microbiology of processed foods, for example--dehydrated foods, is another area of research activity.

New and improved vegetable processing technology is sought in studies of freeze-drying, brining, canning, dehydration, fermentation, hydro-cooling and controlled atmosphere methods. Basic studies deal with new techniques of soaking and preparation, enzyme inactivation and regeneration, fluid flow and heat transfer problems. Special attention is being given to development of high-temperature, short-time methods and the advantages of low-temperature handling of sterilized foods. A comprehensive study of the effects of controlled or modified atmosphere on the biochemical, physical and general quality characteristics of various vegetable products is in progress.

New product research with vegetables is directed toward development of "quick cooking" peas and beans; beet chips; Puerto Rican style soups; snack items; and new sauerkraut products. Methods of processing, product characteristics and storage stability are determined. Some pilot plant research is done, but basic principles relating to composition, quality and functional properties are emphasized. Product characteristics such as sweetness, concentration of individual sugars, rheological properties, softness, water absorption, color and pigment are related to organoleptic properties and consumer acceptability.

The total station scientific research effort devoted to vegetable processing and products is 46.4 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. New and improved dehydrated products

1. Quick-cooking dehydrated vegetable pieces. Dissemination of information and distribution of samples relative to development of explosion-puffed dehydrated vegetables has stimulated industry to evaluate the process. Quick-cooking carrot dice are being produced commercially.

B. New and improved processing technology

1. Equipment development for explosive-puffing. Improvements in locking, releasing and control devices for the new batch gun, combined with a higher surface to volume ratio to expedite heating of the charge, have been developed to shorten the operating cycle, thus increasing production. Increased production, as ultimately obtainable with continuous puffing equipment, will lower costs per unit and result in greater commercial adoption of the process.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

General

Ball, C. Olin, Joffe, Fred M., Stier, Elizabeth F. and Hayakawa, K. 1963. The role of temperature in retaining quality in canned foods. American Society of Heating, Refrigerating and Air-Conditioning Engineers, 5 (6), pp. 93-109.

New and Improved Dehydrated Products

Cording, J., Jr., Eskew, R. K., Sullivan, J. F., and Eisenhardt, N. H., 1963. Explosive puffing process produces quick-cooking dehydrated vegetables. Food Engineering, 35, (6), pp. 52-55.

Komanowsky, M., Eskew, R. K. and Cording, J., Jr. 1964. Pure pumpkin powder. Food Engineering, 36 (5), pp. 107-108.

AREA 8. APPLES AND OTHER FRUITS - PROCESSING AND PRODUCTS

Problem. Lack of knowledge of the nature and quantities of the various chemical constituents and enzyme systems present in fresh fruits, and of the changes these undergo during processing, is a limiting factor in research designed to develop new and improved products and processing techniques. Knowledge is required on the composition and physical structure of fruits and fruit products, with emphasis on substances responsible for color and flavor, vitamins, and other constituents important in determining consumer acceptance and nutritive value of the products. Composition should be studied in relation to variety, stage of maturity, and environmental conditions of growth; and to changes occurring between harvesting and processing, during processing, and in storage and distribution. Recently-developed equipment and techniques have made it possible to isolate, separate, and identify constituents that could not have been handled previously. As basic information is developed, new processing techniques will be applied in the improvement of fruit products, and in more efficient utilization of by-products from fruit processing.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving chemists, biochemists, and chemical engineers engaged in both basic and applied research related to extending the use of fruits in the food processing industries. In the EU program apple products research, and investigations on the chemistry and cell structure of cherries are conducted at Wyndmoor, Pa. Development of rapidly-reconstitutable dehydrated fruit pieces is also underway at Wyndmoor. Contract research on peaches is in progress at Rutgers University, New Brunswick, and on apple texture at the Maryland Agricultural Experiment Station, College Park.

The Federal (EU) scientific effort devoted to research in this area totals 9.5 professional man-years. Of this total, research on chemical composition and physical properties constitutes 3.4 p.m.y., including 0.4 p.m.y. of contract research on apple texture at the Maryland Station. Research on new and improved food products amounts to 3.4 p.m.y., and research on new and improved processing technology amounts to 2.7 p.m.y., including 0.4 p.m.y. of contract research on peach processing at Rutgers.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

State stations are engaged in a comprehensive program involving both basic and applied research on fruit processing and products. Evaluation of fruit varieties and selections is a necessary service for the breeding programs. Additionally the relationships of variety, other production and cultural practices, method of preparation, and processing procedures to quality and utilization of the finished product are determined. Application of mechanical harvesting is spreading rapidly, and makes it necessary to evaluate

how mechanical harvesting affects the utilization of the harvested fruit. Research is also in progress on the identification and characterization of changes associated with post-harvest storage and ripening. The aim is to elucidate the metabolic reactions associated with ripening with a view to their ultimate control. Degradative and structural changes are receiving most careful attention. In some cases the respiratory activity of the fruits is being measured to guide development of holding and packaging requirements.

Work on the chemical composition and physical properties of fruits involves studies of a number of fruits. For example, the non-volatile organic acids and sugars of grapes are being determined. Other studies involve the biochemistry of the color and pigments of fruits. Basic research is in progress on identification of the polyphenols of fruits and the role they play in enzymatic browning. Attempts are being made to isolate and identify the naturally occurring phenolic substances of commercially important fruits which affect the acceptability and stability of fruit juice products, wines, and dried and frozen fruits. Advances in methodology and biochemistry of plant tannins, leucoanthocyanins and related flavonols are being made. The chemical and physical changes of pectic substances before and after processing and during storage are related to texture and reconstitution properties of fruit products. It has been found that ethylene is important in the formation of peroxides and further investigation is concerned with the role of the lipid fraction in ripening of fruits.

Characterization of fruit flavors is being pursued by improved techniques of gas chromatography. Compounds are being identified and effort is being devoted to determining their significance in flavor of the fruit. Flavor variations and off-flavors are being studied also.

Investigation of enzymes specifically involved in the formation of off-flavors in frozen fruits is in progress. Studies of enzyme mechanisms and properties constitute an important fundamental phase of the fruit investigations. Browning problems and control of enzyme reactions are other facets of enzyme systems under study. Some effort is being devoted to synthesis of flavor compounds and pigments through use of extracted natural enzymes. Other analytical work involves determining flavor, texture and nutritional qualities.

The objectives of the microbiology program in fruit utilization vary from determination of the occurrence of certain organisms to study of yeast growth factors important in the wine fermentation. Microbiological spoilage receives careful attention. The use of vitamin K5 and its effect on the various food spoilage organisms is under study. Basic investigations of the ecology, taxonomy and physiology of yeasts and molds involved in food spoilage are made to better understand how spoilage microbes occur in nature and how they may be controlled in food products. A highly specialized study relates to the microbiology of olive fermentation and spoilage. Other research is concerned with the evaluation and enumeration of bacteria found in

frozen fruit products and developing methodology for identifying certain groups.

Research directed to development of new processing technology is a major component of the fruit utilization program. Study of the influence of maturity, post-harvest handling, storage and ripening procedures and processing methods on the quality of canned pear products is an example of a fully integrated project. Comprehensive studies dealing with the thermal processing of a number of fruits are in progress. Basic studies relate to mechanism of heat transfer, the effects of thermal processing and the mechanism of thermal breakdown of various constituents, i.e. fats, proteins, carbohydrates and heat labile vitamins. Methods, equipment and layout of processing lines also receive attention. Methods of freezing, dehydrofreezing, freeze-drying, irradiation and dehydration of fruits are investigated. The effects of the process on organoleptic, physical and chemical characteristics of the fruit are measured. For example, the optimal conditions for dehydrofreezing red cherries are being determined and the dehydrofrozen cherries are being evaluated for use in pies. Investigation of the effect of ultrasonic energy on freeze-drying rate is studied through consideration of the kinetics and mechanisms of energy and mass transfer. Effects of chemicals, hydrocooling, refrigerated storage and controlled atmosphere storage and holding are also under investigation. Because many of the changes in foods relating to processing methods are textural, a fundamental study of the influence of processing on microscopic structure of foods is in progress.

The objective of the product work is to develop new or improved food products. Development of processes or products to improve the utilization of fruits involves work on dehydrated fruits, i.e. prunes; apple juice, apple sauce; frozen fruit pies; apple-fruit juice blends; sherry wines, brined cherries; canned apple slices; low sugar apple jelly; macadamia nuts; peaches and grape products. Factors affecting fluidity, plasticity, consistency, shape, flavor, appearance, texture, physical, chemical and organoleptic properties are being studied.

Other work is concerned with storage of processed fruit items, packaging and containers, methodology for evaluation of fruit products, and the engineering problem of standardization, cooling, sizing and control of atmospheric conditions within packages.

The total research effort on fruit utilization is approximately 57.3 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Chemistry and cell structure of cherries for processing. Study continues on the nature of the changes, instigated by post harvest bruising, that are manifested by scald and other deficiencies in the processing of red tart

cherries. Enzyme preparations which act on cellulose, hemicellulose, starch, pectin and protein were applied to cherries. Microscopic examination of cells indicated no action of enzymes on the cell walls. In radioactive tracer investigations the output of $C^{14}O_2$ from bruised cherries was much greater following injection of C^{14} labeled acetic acid than from C^{14} labeled citric acid.

2. Factors influencing apple texture. In contract research (Maryland Agricultural Experiment Station) the relationship of apple cell wall constituents to texture of apple products is under investigation. No significant differences in cell wall structure of apples before and after storage at 32°F. for periods up to four months could be detected by staining techniques. The rate of softening in storage was found to vary directly with the level of pectin methylesterase activity in the tissue. This activity varies with the variety of apple, being highest in Golden Delicious and lowest in Stayman.

B. New and Improved Food Products

1. "Explosion-puffed" dried fruit pieces. Reports from industry continue to be favorable on tests of dry explosion-puffed apple pieces in cereal and snack items and of reconstituted segments for pies and compotes. In a study comparing varieties, firm-fleshed apples such as York Imperial were rated best for the process.

Explosive-puffed blueberries have been successfully tested in bakery products.

2. Improved apple cider. The formation of hydroxymethyl furfural (HMF), which accompanies the darkening of pasteurized cider on storage, was found to be accelerated by heat and acid and to increase with concentration of the juice. It appears that sucrose does not form HMF directly, but that HMF is formed after the sucrose is hydrolyzed to fructose and dextrose. Diethyl pyrocarbonate in proportions of 25 parts per million and above reduces the microbial count of fresh apple cider to a relatively low level. Combinations of this compound with potassium sorbate were superior to either preservative alone.

C. New and Improved Processing Technology

1. Processing characteristics of eastern peaches. Several promising new varieties of peaches were selected for further evaluation as a result of contract research (New Jersey Agricultural Experiment Station) on processing characteristics of 100 varieties. A common characteristic of the selected new varieties was an outstanding flavor. In general, the processed freestone peaches had better color than clingstone varieties.

2. Equipment development for explosion-puffing. Firing the "gun" at a slight downward angle emptied the chamber effectively, but aggravated the

problem of collecting the fragile pieces of fruit. A 25-foot long receiver-conveyer was installed, and further modifications have been made at the receiving end to minimize the problems of deceleration and collection.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

Gaston, H. P. and Levin, J. H. 1964. Effect of recurrent bruising on the processing of red tart cherries. Mich. Agr. Expt. Sta., Research Report No. 4, 5 pp.

New and Improved Food Products

Eisenhardt, Nelson H., Eskew, Roderick K. and Cording, James, Jr. 1964. Explosive-puffing applied to apples and blueberries. Food Engineering, 36, No. 6, pp. 53-55.

New and Improved Processing Technology

Gaston, Harold P., Levin, J. H., Hedden, S. L. and Whittenberger, R. T. 1963. Mechanical harvesting of cherries. The results obtained in 1962 are promising. A panel discussion. Michigan State Horticultural Society. Annual Report 92, pp. 86-98.

Harrington, W. O. and Hills, Claude H. 1964. Lye peeling of apples improved by wax solvents. Food Technology, 18, pp. 375-376.

Whittenberger, R. T., Gaston, H. P., Hedden, S. L. and Levin, J. H. 1963. Comments on mechanical harvesting of cherries in 1962. Great Lakes Cherry Producers Marketing Cooperative, Inc. 1962 Progress Report, pp. 10-19.

AREA 9. TOBACCO - COMPOSITION AND PROCESSING

Problem. Although neither food nor fiber, tobacco nevertheless is grown on about a million acres, and in seven states provided more farm cash receipts than any other field crop in 1960. The farm value is about $1.3 billion. This crop is unique in that it yields about $3.1 billion in Federal and State taxes. Several serious difficulties plague the industry, among them the lack of genuine scientific knowledge of the composition of tobacco and tobacco smoke, which can be used to solve many industrial problems. By knowing the chemical factors in the leaf which result in an acceptable smoke, it would become possible to predict accurately the usefulness of a particular tobacco for smoking purposes, and thus solve a long-standing industrial problem. Methods could also be devised to expedite current time-consuming and erratic methods of fermenting cigar tobacco. Finally, more selective studies on tobacco smoke could be made, including the origin and fate of leaf constituents during burning, the formation of substances having physiological effects, and ways of producing smoke of diverse composition.

USDA AND COOPERATIVE PROGRAM

The Department's continuing program involving chemists engaged in basic and applied studies of the chemical composition of tobacco leaf and smoke was directed to better understanding of and improvement in tobacco quality, and to improvement in tobacco processing technology. The direction of this program has been changed so as to place special emphasis on constituents which have been implicated in the smoking and health problem. The program now includes: (a) fundamental studies on the isolation and identification of chemical substances in cigarette leaf and smoke, (b) investigations on the combustion products of known constituents of cigarette tobaccos, and (c) studies on the elimination of deleterious substances in cigarette smoke through alteration of burning pattern and by selective filtration.

The Federal work is conducted at Wyndmoor, Pa., and Durham, N. C., and totals 10.7 professional man-years, 6.7 of which are devoted to study of the composition of tobacco smoke, mainly cigarette smoke, and 2.0 to composition of oxidation products and related substances, primarily of cigarette leaf. A research contract on chemical investigations of the neutral resins of tobacco leaf involving 2 professional man-years per year is under way at the Research Triangle Institute, Durham, N. C. In addition, the Cigar Manufacturers' Association of America supports a research program on cigar smoke at Wyndmoor, that is the equivalent of 2.0 professional man-years.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

A modest program of research related to the utilization of tobacco is conducted by the stations. Considerable effort is devoted to determining the effect of agronomic practices on the curing and fermentation process. The effect of chemical and microbiological changes during curing and fermentation

are also being investigated. In addition, development of analytical techniques for evaluation of tobacco characteristics receives some attention.

Study of curing methods, curing environments and the use of machines and machine methods in tobacco harvesting and processing is expanding. Research is also directed to the determination of the physical and chemical properties of tobacco; the composition of cigarette tobacco; and the chemistry of tobacco aroma and its source. In the study of tobacco aroma, major effort is devoted to collection of the volatile oils and their fractionation and identification.

The objective of work in progress at the Puerto Rico station is to determine standards of quality in tobacco and how best to carry out the fermentation and curing of tobacco leaves for cigar manufacture.

The total program involves 11.4 p.m.y.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Composition of Tobacco Smoke

1. Cigarette smoke. Further advances have been made in identifying the volatile constituents of cigarette tobacco leaf and smoke and in relating their presence to aroma and flavor variations. Four acids (n-butyric, n-heptylic, n-caprylic and n-pelargonic) were reported in cigarette tobacco leaf for the first time. Evidence was also obtained for the presence of the bases nicotinonitrile and metanicotine, and details were worked out for the isolation of 3-vinylpyridine from smoke. A total of thirty-two volatile bases were detected, the principal ones being pyridine, picolines, 3-vinylpyridine and nicotine.

The contribution of the various tobacco types to the flavor and character of blended cigarette tobacco was ascertained by a study of certain potentially flavorful volatile components in the smoke. An outgrowth of this was the development of a process to replace the Turkish tobacco in American cigarettes with domestic leaf to which has been added a mixture of isovaleric and β-methylvaleric acids which occur in comparatively large amounts in Turkish tobacco. The process merits consideration by industry, because tests indicate that the smoke flavor is indistinguishable from that of cigarette blends containing 5-20% Turkish tobacco.

2. Cigar smoke. The major volatile bases in cigar smoke have been isolated and identified as pyridine, 3-ethylpyridine, isomeric picolines, isomeric lutidines, 3-vinylpyridine, nicotine, nornicotine, myosmine and 2,3'-dipyridyl. The smoke of three commercial cigar brands contained similar amounts of the principal volatile bases (pyridine, picoline, 3-vinylpyridine). The smoke of typical domestic tobaccos contained less of the alkaloids (nicotine, especially) and more of the volatile bases (pyridine) than the smoke of "green" Brazilian tobaccos, reflecting the degree of fermentation.

Sufficient analytical data were obtained to compare the compositions of cigar and cigarette smoke on the basis of microgram per puff. The volatile acids were qualitatively similar, although cigarette smoke contained greater amounts of the C_2 - C_6 acids which are believed to contribute aroma to cigarette leaf and flavor to its smoke. The volatile bases were also similar and generally within the same range except that cigarette smoke contained higher concentrations of the alkaloids (nicotine, nornicotine-myosmine, etc.). Preliminary work on the neutral fractions indicates that cigar smoke contains at least 75 such components which may prove comparable to the neutral compounds of cigarette smoke.

B. Composition of Oxidation Products and Related Substances.

Fractionation studies to resolve the highly complex nature of the oxidation products of tobacco have led to the isolation of an interesting mixture of high molecular weight compounds from Turkish tobacco. This fraction accounts for a significant part of the leaf weight (about 3%) and resembles the dark pigments recently isolated by other workers and described as responsible for tobacco color. Preliminary work with this mixture showed that on hydrolysis it forms rutin, chlorogenic acid, amino acids and iron; on pyrolysis it produces more than 10 components which superficially resemble the pyrolytic products of rutin and other polyphenols. The nature of these breakdown products suggests that the newly isolated pigments may have a role in the generation of harmful and/or flavorful substances during burning.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Composition of Tobacco Smoke

Burdick, D., Schmeltz, I., Miller, R. L. and Stedman, R. L. 1963. Composition studies on tobacco. XIV. Steam-volatile, neutral substances in various types and grades. Tobacco Science, 7, pp. 97-100.

Burdick, D. and Stedman, R. L. 1963. Composition studies on tobacco. XV. Steam-volatile, neutral substances in smoke from blended and unblended cigarettes. Tobacco Science, 7, pp. 113-117.

Osman, S. R., Schmeltz, I., Higman, H., and Stedman, R. L. 1963. Volatile phenols of cigar smoke. Tobacco Science, 7, pp. 141-143.

Schmeltz, I., Miller, R. L. and Stedman, R. L. 1963. Gas chromatographic study of the steam-volatile fatty acids of various tobaccos. J. Gas Chromatog., 1, pp. 27-28.

Schmeltz, I., Stedman, R. L. and Miller, R. L. 1963. Composition studies on tobacco. XVI. Steam-volatile acids. J. Assoc. Offic. Agr. Chemists, 46, pp. 779-784.

Stedman, R. L. 1963. Arome, saveur et composition chimique du tabac et de la fumee de cigarette. CORESTA Bulletin d'information, No. 4, pp. 11-23.

Stedman, R. L., Burdick, D. and Schmeltz, I. 1963. Composition studies on tobacco. XVII. Steam-volatile acidic fraction of cigarette smoke. Tobacco Science, 7, pp. 166-169.

General

Ogg, C. L. 1964. Report on tobacco. J. Assoc. Offic. Agr. Chemists, 47, p. 49.

Ogg, C. L. and Cundiff, R. H. 1963. Determination of chlorides in tobacco. J. Assoc. Offic. Agr. Chemists, 46, pp. 415-418.

Ogg. C. L. and Cundiff, R. H. 1963. Determination of potassium in tobacco. J. Assoc. Offic. Agr. Chemists, 46, pp. 413-415.

Stedman, R. L. and Miller, R. L. 1963. Some pitfalls in studies related to gas chromatography. J. Chromatog., 11, pp. 409-411.

Steyermark, A., Alber, H. K., Aluise, V. A., Huffman, E. W. D., Jolley, E. L., Kuck, J. A., Moran, J. J., Ogg, C. L. and Pietri, C. E. 1963. Report on recommended specifications for microchemical apparatus. Van Slyke apparatus.

Microchemical J., 7, pp. 233-246.

AREA 10. MAPLE SAP AND SIRUP - PROCESSING AND PRODUCTS

Problem. The extensive unused stands of sugar maple trees are largely located on land that is of marginal value to agriculture, areas commonly devoted to small-scale dairy farming. Since only a small percent of the available sugar maple trees are presently tapped for sirup, and we are importing some 50% of the sirup consumed in the United States, untapped sugar maples represent a good potential source of increased cash income for farmers in these areas. Based largely on recent research carried out in the Department and the State Experiment Stations, the methods of collecting and processing sap into sirup are being streamlined with resulting greatly increased efficiency and resulting greater hourly return to the sirup producer for his labor. The advent of tube collection of sap and the central evaporator plant promise to still further reduce the number of man-hours required to produce a gallon of finished sirup. Under proper conditions, maple sirup can be a seasonal crop of per acre value equal to or exceeding that of other farm products. While the results of previous research, such as establishing the great importance of sanitary collection of sap, have contributed to "modernization" of the industry, much more information is needed so that all operations for the production of high-quality maple sirup and other maple products can be conducted in a predictable, efficient manner. Not only can the low income farms be greatly benefited, but the existing maple industry in 14 states can be put on a higher economic plane and modernized to be made competitive with other crop and livestock farming to bring about an improved land use.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving chemists, biochemists and microbiologists. These scientists are engaged in both basic and applied research in investigations concerned with the problems of improving sap handling and processing, producing high-quality maple sirup, and developing new outlets for all maple products while lowering the cost of the product. This work is conducted at Wyndmoor, Pa.

The Federal scientific effort devoted to research in this area totals 5.3 professional man-years. Of this number 2.6 are devoted to study of the chemical composition and physical properties of maple sap and sirup, 1.2 to microbiology of maple products and 1.5 to new and improved food products and processing technology.

In the research work cooperation is maintained with personnel of the Federal Extension Service in maple-producing states and with Cornell University.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

A small research program on maple sap and sirup is in progress. Factors affecting the periodic sap flow in sugar maple are being studied by use of

labeled isotopes. Effects of taphole treatment and collection methods are also being evaluated. Since the entry of the central evaporator into maple sirup operations, a study concerned with the economic feasibility of central evaporator processing has been completed. A good quality product may be obtained and analysis indicates a good potential for central processing.

Research on maple products production is currently concerned with sap flow, sap collection and sap dumping in systems using plastic tubing. Further investigation of the various sirup making operations and their effect on sirup quality is being pursued.

Some attention is being directed toward marketing systems for maple products and study of the potential demand for maple products in outlets located in heavily populated areas.

The number of professional man-years devoted to maple products utilization is 0.8.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

Investigations on maple sap and sirup to obtain information on which to base improved processing techniques for making better maple products at lower cost are being carried out in three different areas: (1) Formation of sugar sand in maple sirup, (2) Composition of maple flavor, and (3) Fermentation studies. A fourth study, storage of maple sap, will be undertaken through contract research as soon as a contract can be negotiated with a suitable processor.

A. Processing

1. Maple sugar sand formation. Studies in this area have been carried out through a research contract with the Ohio Agricultural Experiment Station at Wooster. The experimental work under the contract has been completed and will be terminated on receipt of the final report, which will be published as an Ohio Agricultural Extension Bulletin. Statistical analysis of the data shows: (1) A high correlation between sugar sand formation and calcium ion concentration of the sap, (2) Sugar sand formation is related to elevation and aspect (north, south, east or west exposure) of the sugar bush, i.e., sugar bushes at high elevation and southern exposure produce most sugar sand, whereas low elevation and western exposure produce least amounts of sugar sand, (3) High precipitation in the spring favors sand formation, and (4) Sugar sand formation tends to increase as sap flow season progresses.

The information obtained in this study dealing with the factors responsible for sugar sand formation and its character should be of considerable value in devising improved processes for making maple sirup. The study has also generated great interest in maple in Ohio as one of the natural resources of the State.

B. Composition Studies

1. Composition of maple flavor. The chemical nature of the maple flavor constituents that have been identified so far (vanillin, coumarin, syringaldehyde, coniferyl aldehyde, dihydroconiferyl alcohol and 2,6-dimethoxybenzoquinone) suggests their possible formation from soluble lignin fractions. Additional evidence was obtained to support this concept in that vanillin was produced by oxidation of ether-precipitated ligneous material from sample sap.

Since maple sirup is prized because of its characteristic flavor, additional information on the individual components of its flavor and of the mechanism of its formation from precursors in the sap will be of great value in preparing of maple products of improved quality. This work will be continued along present lines. Studies will also be continued on isolating and characterizing flavor precursors in the sap. It is also of considerable importance to develop specific methods for measuring formaldehyde in sap. Use of non-specific methods which would measure carbonyl groups (sugar breakdown products) as formaldehyde might give erroneously high values that could exceed limits of values set by the Food and Drug Administration. These limits were set by F.D.A. because of the use of paraformaldehyde as a tap hole sterilant.

C. Fermentation Studies

1. Production of acceptable maple sirup from buddy sap. Production of acceptable sirup from buddy sap would enable sap producers and processors to eliminate or reduce economic losses from this factor. The use of paraformaldehyde pellets in tap holes has extended sap flow into warm weather when buddy sap can be expected, and the possibility that large amounts of buddy sap will be collected at central evaporator plants places increased significance on developing procedures for converting buddy sap into an acceptable product.

Previous investigation has shown that controlled fermentation of buddy sap with the organism Pseudomonas geniculata removes the buddy flavor principle. Progress has been made in applying the procedure to buddy maple sirup. Fermentation of sirup diluted with about three volumes of water eliminated or markedly reduced the disagreeable flavor and odor of the buddy sirup. Studies on the organisms that produce slime or gums in buddy sap should develop information that would be useful in devising methods that will reduce or eliminate their effect on the sirup.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Composition

Stinson, E. E. and Willits, C. O. 1963. Separation of caramel color from salts and sugar by gel filtration. J. Assoc. Offic. Agr. Chemists, 46, pp. 329-330.

Underwood, J. C. 1963. Quick test for "buddy" flavor in maple sirup. U. S. Agricultural Research Service, ARS 73-42, 4 pp.

Underwood, J. C. and Filipic, V. J. 1963. Gas chromatographic identification of components in maple sirup flavor extract. J. Assoc. Offic. Agr. Chemists, 46, pp. 334-337.

Underwood, J. C. and Willits, C. O. 1963. Research modernizes the maple sirup industry. Food Technology, 17, pp. 1380-1382, 1384-1385.

Willits, C. O. 1964. Report on maple products. J. Assoc. Offic. Agr. Chemists, 47, pp. 67-68.

Willits, C. O., Costilow, R. N., and Underwood, J. C. 1963. Formaldehyde in maple sirup: An adaptation of the Nash method. J. Assoc. Offic. Agr. Chemists, 46, pp. 331-333.

Willits, C. O. and Stinson, E. E. 1963. Methods for the determination of carboxyl groups. In "Handbook of Analytical Chemistry," edited by L. Meites, Sec. 12-69 to 12-84, McGraw-Hill, New York.

Fermentation Studies

Wasserman, A. E. 1963. Collaborative study of the method for counting microorganisms in maple sirup. J. Assoc. Offic. Agr. Chemists, 46, pp. 321-328.

Wasserman, A. E. Microbiology and sanitation in the sugarbush and sugarhouse. 1963. U. S. Agricultural Research Service, ARS 73-41, 18 pp.

AREA 11. HONEY - PROCESSING AND PRODUCTS

Problem. Essential pollination of over fifty crops depends almost completely on the honeybee because of changes in agricultural practices over recent years. The importance of the beekeeper in our economy is thus emphasized. As most of his income results from the sale of honey, the beekeeper not only is subject to uncertainties of crop and weather, but must also contend with disease, losses of crop and bees from insecticides, rising costs of needed equipment and materials, lack of trained help, all compounded with uncertain and depressed markets for honey. Because of the relatively small size of operations and the scattered nature of the industry, the honey producer is out-researched, out-promoted and out-advertised by competing sweetening agents. Improved processing methods and equipment, better control of product quality, outlets for lower-grade honey, stable export markets, increased use of honey, both in food manufacture and the home, and increased industrial use of byproducts are all needed to provide an expanding market and encourage the beekeeping industry.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long term program involving chemists and biochemists engaged in both basic studies and the application of known methods and principles to the solution of honey producers' problems. This work is done at Wyndmoor, Pa.

The Federal scientific effort devoted to research in this area totals 4.0 professional man-years, all devoted to research on enzymes in honey.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

Three stations are conducting research on honey utilization. Previous research under one project has involved study of the chemical and physical properties of California honeys. Methods of determining diastase content, effect of heat on the enzymes and effects of repellents on bees and on honey quantity are currently being investigated. Discovery and identification of those special ingredients in honey that cause either favorable or unfavorable reactions in the product or in honey processing is the objective of a second study. Under another study, equipment is being designed and evaluated for suitability for rapidly heating, straining and cooling honey. Causes of undesirable flavor are also being studied.

Total research effort on honey is approximately 2.7 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Enzymes

Because of the need for more complete information on the components of honey,

which includes its enzymes, and the fact that some European countries use enzyme content as an index of purity or quality, studies on more complete characterization of the enzymes of honey have been stressed. A process was developed for purifying honey glucose oxidase and improvements were made in assay methods for this enzyme. Glucose oxidase was found to oxidize β-glucose about six times faster than α-glucose. Mannose is oxidized only slowly by this enzyme. Invertase and amylase activities of honey have been separated by gel filtration. Wide variability in light sensitivity of inhibine among different honey samples is attributed in part to a newly discovered sensitizing material. Variability in amount and light-sensitivity and heat stability of inhibine tends to invalidate the use of this property as a quality index by European importers. Additional knowledge along these lines will permit more critical evaluation of methods used by European importers of American honey, based on enzyme values as quality indexes. It also will place us in a better position to meet the requirements of importers of our honey from the standpoint of enzyme activity.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

Enzymes

Schepartz, A. I. and Subers, M. H. 1964. The glucose oxidase of honey. I. Purification and some general properties of the enzyme. Biochimica et Biophysica Acta, 85, pp. 228-237.

White, J. W., Jr. 1964. Diastase in honey: The Schade method. J. Assoc. Offic. Agr. Chemists, 47 pp. 486-488.

White, J. W., Jr., and Subers, M. H. 1963. Studies on honey inhibine. II. A chemical Assay. J. Agr. Res., 2, pp. 93-100.

White, J. W., Jr., Subers, M. H., and Schepartz, A. I. 1963. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide, and its origin in a honey glucose-oxidase system. Biochimica et Biophysica Acta, 73, pp. 57-70.

General

White, J. W., Jr. 1964. Dextrose determination in honey: A rapid photometric procedure. J. Assoc. Offic. Agr. Chemists, 47, pp. 488-491.

White, J. W., Jr., Subers, M. H., and Kushnir, I. 1963. How processing and storage affect honey quality. Gleanings in Bee Culture, 91, pp. 422-425.

White, J. W., Jr., Farrar, C. L., Moeller, F. E., and Woodrow, A. W. February 25, 1964. Method of repelling bees with acetic and propionic acids. U. S. Patent 3,122,473.

AREA 12 - REPLACEMENT CROPS - UTILIZATION POTENTIAL - EASTERN REGION

Problem. Farmers could achieve economic use of their land if new and profitable crops were available that would have different end-uses than crops presently grown. For example, it would be advantageous to develop a new oilseed crop yielding unique fatty acids that could find industrial use in applications for which acids from presently available domestic oilseed crops are unsuitable.

To develop a new crop, three basic steps are involved: (1) survey of wild plants, in cooperation with plant scientists, to identify those having both potentially valuable components and promising agronomic potential for use in the U.S.; (2) detailed physical and chemical characterization of components and basic research to obtain clues to likely end-uses; (3) selection of the most promising species, followed by additional utilization research to explore uses and demonstrate industrial potential and by additional agronomic research to establish proper cultural practices and select the best strains and varieties.

Only after these steps have been successfully accomplished can a proposed new crop be offered to agriculture and industry for introduction and development. Obviously, a program of this type is a long-range one. Yet such long-range research is necessary if agriculture and the nation are to benefit from availability of the best practical crop plants.

To achieve this objective, survey and characterization work needs to be greatly increased, since the greater the number of species examined, the greater will be the opportunities for finding plants meeting the criteria of high utilization and agronomic potential. Work of the Department has already revealed several promising sources of new potentially valuable water-soluble gums, pulp fibers, and oils containing unique fatty acids such as hydroxy unsaturated acids, capric acid, epoxy acids, and unusual long-chain fatty acids. In order to demonstrate the potential of these new materials, further work is required on their physical and chemical properties and reactions, on processing to obtain maximum recovery from source plants and on byproducts from processing, such as oilseed meals.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving chemists engaged in both basic and applied studies directed to the development of profitable new crops.

At Wyndmoor, Pa., work on new crops totals 5.5 professional man-years. The research is concerned with a study of the oil obtained from the seed of the Indian ironweed (Vernonia anthelmintica), in cooperation with the Northern Utilization Research and Development Division, the Crops Research Division

and the Western Utilization Research and Development Division. The oil contains epoxy fatty acids, potentially useful industrial chemicals.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

Discovery and preservation of valuable plant germ plasm is a continuing objective of the station program in new crops. Much of the research in this area is being done via four regional projects and in cooperation with regional centers. A large portion of the work is cooperative with USDA. Each year many plant introductions are grown and evaluated. Annual and perennial crops possessing potential for industrial or agricultural use are further evaluated for agronomic and chemical qualities. These include crops for paper pulp, drugs, insecticides, polysaccharide gums, and oils rich in acids of unusual structure. Assay of native and introduced tropical plants for products of economic value receives special attention.

Basic aspects of this program involve study of the biochemical and physiological basis for differences in crop plants. Attempts are made to determine if differences in biochemical or physiological processes can be associated with particular factors related to quality. Information concerning carbohydrate transformations is sought through study of carbohydrate formation and enzyme mechanisms. Horticultural specialty crops are gaining in importance. A number of studies are underway to facilitate rapid development of this industry.

The total scientific effort devoted to replacement crops is 9.2 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

Progress in the development of Vernonia anthelmintica seed oil as a source of an epoxy-containing oil has been made in the following areas, (1) commercial extraction of 3000 lbs of seed obtained from Pakistan and 750 of domestically grown seed has been carried out through cooperation with an industrial plant under our supervision; (2) over 400 samples of seeds, grown in collaboration with the New Crops Research Branch of ARS have been analyzed; (3) data have been obtained on the efficiency of various solvents used for purifying trivernolin, the chief component of Vernonia oil; (4) domestic seed has been upgraded by an air-flotation procedure that separates the light from the heavy seed; (5) ion-exchange procedures have been employed to remove undesirable fatty acids from Vernonia oil; and (6) separation of unsaponifiable material from the oil by a method previously worked out is being applied to the commercial extracted oil, and the unsaponified material will be evaluated for use in chick feeding. A result of particular interest is that seeds grown above the 39th parallel show low oil yields and the oil is high in free fatty acids and low in epoxy oxygen, indicating immature seeds.

Pronounced commercial interest in Vernonia continues, and industry will be supplied with samples shortly to meet the many requests for Vernonia materials for evaluation purposes. At least one industrial agency has grown

Vernonia on a small scale experimental basis. There is need for more agronomic information, and knowledge of adequate harvesting methods for the seed. Progress in plastic evaluation studies supports the need for commercial evaluation of Vernonia oil and its derived products.

Since processing techniques have been developed and it has been shown that soya extraction plants can be used for Vernonia without extensive modification of equipment, more attention can be given to the preparation and evaluation of modified products and derivatives of Vernonia oil, trivernolin and 12,13-dihydroxy oleic acid. Synthesis of requested vernolic acid derivatives for pharmaceutical studies and further composition studies will be carried out.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

Utilization of Oilseeds Containing Epoxidized Oils

Krewson, C. F., and Luddy, F. E. 1964. Vernonia Anthelmintica (L.) Willd. Highly purified epoxy components from seed oil. J. Am. Oil Chem. Soc., 41, pp. 134-136.

Riser, G. R., Hunter, J. J., Ard, J. S., and Witnauer, L. P. 1963. Epoxy acids and salts as stabilizers for vinyl plastics. Soc. Plastics Engrs. J. 19, (8), pp. 1-6.

Sampugna, J., Jensen, R. E., Parry, R. M., Jr., and Krewson, C. F. 1964. Lipolysis of trivernolin by pancreatic lipase. J. Am. Oil Chem. Soc. 41, pp. 132-133.

Scott, W. E., Krewson, C. F., Luddy, F. E., and Riemenschneider, R. W. 1963. Vernonia anthelmintica (L.) Willd. Enzyme studies. Conversion of epoxy-oleic acid to (+) -threo-12,13-dihydroxyoleic acid. J. Am. Oil Chem. Soc., 40, pp. 587-589.

Scott, W. E., Krewson, C. F., and Riemenschneider, Roy, W. June 30, 1964. Preparation of (+)-threo-12,13-dihydroxyoleic acid. U. S. Patent 3,139,387.

Precursors for Steroidal Hormones

Levine, S. G., and Wall, M. E. March 10, 1964. Addition of hypohalites to unsaturated steroids. U. S. Patent 3,124,570.

LINE PROJECT CHECK LIST -- REPORTING YEAR 63 to 64

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
E6 2	Milk Products Utilization Investigations. Program Leadership	Wyndmoor, Pa. and Washington, D. C.		
E6 2-74*	Chemistry of flavor changes during processing and storage of dry milk	Washington, D. C.	Yes	1,2-A-2
E6 2-77*	Improvement in the quality of Cheddar cheese	Washington, D. C.	Yes	1,2-D-2
E6 2-79*	Factors affecting flavor score and storage stability foam-dried milk	Washington, D. C.	Yes	1,2-A-1
E6 2-81(Rev.)	Removal of radioactive contamination from milk	Beltsville, Md.	Yes	1,2-E
E6 2-82(C)*	The chemical nature of stale flavor in aged sterile milk	Urbana, Illinois	Yes	1,2-A-2
E6 2-83(C)	Chemistry of Cheddar cheese flavor	Columbus, Ohio	Yes	1,2-A-3
E6 2-84	A laboratory and pilot plant study of the effect of chemical additives on the storage stability of evaporated milk	Washington, D. C.	Yes	1,2-B-1
E6 2-85(Rev.)	Interactions of milk proteins in solution	Wyndmoor, Pa.	Yes	1,2-C-3
E6 2-87	Methods for making fat-free and low-fat cheese	Washington, D. C.	Yes	1,2-D-2
E6 2-88	Physico-chemical studies of factors influencing milk fat-plasma emulsion stability	Washington, D. C.	Yes	1,2-B-2
E6 2-89(C)	Development of improved techniques for evaluating importance of flavors in new concentrated milk products	Corvallis, Oregon	Yes	1,2-A-1
E6 2-90	Improvement of concentrated whole milk products	Washington, D. C.	Yes	1,2-C-3
E6 2-91**	Properties of components of milk related to physical changes during processing and storage	Wyndmoor, Pa.	Yes	1,2-C-3
E6 2-92	Development of increased food outlets for nonfat milk solids	Washington, D. C.	Yes	1,2-D-3
E6 2-93	Development of a commercially feasible process for preparing a beverage quality dry whole having adequate shelf life	Wyndmoor, Pa.	Yes	1,2-B-2
E6 2-94(C)	Effects of nonfat dry milk on bread yeast fermentation	Madison, Wis.	Yes	1,2-D-3
E6 2-95 (Gr.)	Increased protein stability of evaporated milk: study of calcium phosphate-casein micelles	Columbus, Ohio	Yes	1,2-B-1
E6 2-96	Improving the flavor stability of anhydrous milk fat	Washington, D. C.	Yes	1,2-A-1
E6 2-97	The chemistry of bacterial spores	Washington, D. C.	Yes	1,2-C-4
E6 2-98	Studies on stale flavor in sterile milk and development of means to prevent its formation	Washington, D. C.	Yes	1,2-A-2
E6 2-99	Improved sterile whole milk concentrates: the production of reversible sol-gel transformations in high solids sterile concentrates	Washington, D. C.	Yes	1,2-B-1
E6 2-100(C)	Removal of radioactive strontium from milk on a commercial scale	Springfield, Mo.	Yes	1,2-E
E6 2-101***	Ribosomal nucleic acids	Wyndmoor, Pa.	Yes	1,2-C-5
E6 2-102 (C)***	Heat stability of individual milks	St. Paul, Minn.	Yes	1,2-C-3
E6 2-103***	Enzyme studies relating to milk	Wyndmoor, Pa.	Yes	1,2-C-6
E6 2-104***	Casein properties	Wyndmoor, Pa.	Yes	1,2-C-3
E6 2-105 (Gr.)***	Physical changes in milk and milk concentrates associated with steam injection and bubble collapse	Raleigh, N. C.	Yes	1,2-B-1

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area and Subheading
E6 2-107 (C)***	Relation of milk fat composition, particularly fat and protein, to dietary of cow	College Park, Md.	No	
E6 2-109 (Gr.)***	Study on the desirable flavors of butter: Isolation and identification of specific flavor contributing compounds and their precursors	Corvallis, Oregon	No	
E6 2-113***	Improvement of dried whole milk	Washington, D. C.	No	
UR-A7-(60)-5	Milk coagulating enzymes	India	Yes	1,2-C-8
UR-A7-(60)-11	Sulfur compounds in relation to flavor and stability of milk	India	Yes	1,2-A-2
UR-A7-(60)-13	Export outlet for nonfat dry milk as additive to buffalo milk in cheese manufacturing	India	Yes	1,2-C-8
UR-A7-(60)-16***	Phosphoproteins of milk	India	Yes	1,2-C-8
UR-A7-(60)-22	Proteose-peptone fraction of milk	India	Yes	1,2-C-8
UR-E8-(60)-1	Growth-promoting factors for lactic acid bacteria	Finland	Yes	1,2-A-3
UR-E8-(60)-16***	Dietary factors controlling flavor in milk	Finland	Yes	1,2-A-2
UR-E9-(60)-46	Nonprotein nitrogenous constituents of milk	France	Yes	1,2-C-8
UR-E9-(60)-47	Proteolytic activity of rennin on casein	France	Yes	1,2-C-8
UR-E9-(10,60)-80	Structure of nucleic acids	France	Yes	1,2-C-8
UR-E10-(60)-3***	Surface changes in fat globules of dried whole milk	West Germany	Yes	1,2-B-2
UR-E21-(60)-7	Increasing vitamin B in cheese	Poland	Yes	1,2-D-2
UR-E21-(60)-21	Mechanisms of cheese ripening process	Poland	Yes	1,2-D-2
UR-E25-(60)-18	Protein destabilization in frozen milk	Spain	Yes	1,2-B-1
UR-E26-(60)-9***	Methods for purification of protein complexes	Sweden	Yes	1,2-C-8
UR-E29-(60)-31*	Microorganisms in dairy products	United Kingdom	Yes	1,2-A-3
UR-E29-(60)-41	Studies on selected enzymes of milk	United Kingdom	Yes	1,2-C-8
UR-S3-(60)-10	Active site of trypsin	Brazil	Yes	1,2-C-8
UR-E15-(60)-16*	Food products from milk and fruit concentrates	Italy	No	

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area and Subheading
E6 2-106(C)***	Fraction of milk fat for specific food uses	Not yet determined	No	
E6 2-108(Gr.)***	Flavors and their precursors in milk derived from pasture or dry feeding practices	Not yet determined	No	
E6 2-110(C)***	Commercial scale development of low-fat Cheddar-type cheese	Not yet determined	No	
E6 2-111(Gr.)***	Lactones, methyl ketones and their precursors in milk products: effects on off-flavors and development of procedures for their control	Not yet determined	No	
E6 2-112(C)***	Fluid milk from nonfat dry milk and butteroil	Not yet determined	No	

* Discontinued during report year.
** Superseded during report year by E6 2-103 and E6 2-104.
*** Initiated during report year.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area and Subheading
E6 5	Meat Utilization Investigation. Program Leadership			
E6 5-17(Rev.)**	Studies of chemical and microbiological factors involved in the processing and storage of meats	Beltsville, Md.	Yes	3-B
E6 5-18(C)*	A study of meat pigments and their relationship to lipid oxidation and stability of frozen, cured, and uncured meats	Tallahassee,Fla.	Yes	3-B
E6 5-19(Rev.)	Identification of substances responsible for flavor and aroma in meat	Beltsville, Md.	Yes	3-D
E6 5-20(Rev.)	Chemical reactions involved in meat curing	Beltsville, Md.	Yes	3-C
E6 5-21(C)	Investigations on selected characteristics of connective tissue and modification to improve meat tenderness	Baton Rouge,La.	Yes	3-C
E6 5-22(Rev.)	Improving the quality of meat through studies of microorganisms capable of growth near $0^{\circ}C$. with particular emphasis on their nutrition and enzymatic activity	Beltsville, Md.	Yes	3-A
E6 5-23	Improving the flavor of cured meats through a study of the interrelationships of temperature, curing substances, microbial metabolism, and microbial mutation rates	Beltsville, Md.	Yes	3-A
E6 5-24(Rev.)	Studies of the physical and chemical characteristics of meat to obtain basic information needed in developing improved methods of meat processing. Meat protein composition and distribution in relation to tenderness and juiciness	Wyndmoor, Pa.	Yes	3-C
E6 5-26(C)	A comprehensive study of fungi associated with meat processing and flavor development in order to develop new products of distinctive flavor characteristics	Ames, Iowa	Yes	3-A
E6 5-27	Improving smoked meat products by identifying the substances present in wood smoke that either directly or through reactions contribute to the flavor and aroma of smoked meats	Wyndmoor, Pa.	Yes	3-D
E6 5-28	Development of new or improved meat processing methods and of new meat products	Wyndmoor, Pa.	Yes	3-C
E6 5-30(C)**	Development of new smoke meat products that will apply new knowledge and provide new avenues for meat utilization	E. Lansing,Mich.	No	
E6 5-31(C)**	Development of new frozen meat products and determination of their time-temperature-tolerance relationships in order to develop new avenues for the utilization of meats	Columbia, Mo.	No	
E6 5-32(Gr.)**	Studies of chemical and microbiological factors involved in the freezing of meat: The relationship of the amounts and ratios of heme pigments to oxidative rancidity	Tallahassee,Fla.	No	
UR-E8-(60)-14	Influence of fats on flavor and aroma of dry sausage	Finland	Yes	3-B
UR-E21-(60)-24	Antioxidant components of wood smoke used in meat curing	Poland	Yes	3-C
UR-E29-(60)-15	Enzymes attacking animal connective tissue	United Kingdom	Yes	3-C
UR-E29-(60)-70**	Specific reducing systems in pork muscle	United Kingdom	Yes	3-C

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
UR-E8-(60)-4*	Effect of microorganisms on sausage flavor	Finland	Yes	3-B
UR-E15-(60)-13*	Studies on beef canning	Italy	No	

* Discontinued during report year.
** Initiated during report year.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area of Subheading
E6 3	Animal Fats and Oils and Special Products Utilization Investigations. Program Leadership.			
E6 3-(55)(Rev.)	Long-chain fat derivatives for polymer modification	Wyndmoor, Pa.	Yes	4-B-1
E6 3-56*	Phosphorus, sulfur, nitrogen and oxygen-containing fat derivatives	Wyndmoor, Pa.	Yes	4-D-3;4-B-2
E6 3-58(Rev.)	Soap-detergent combinations based on animal fats	Wyndmoor, Pa.	Yes	
E6 3-59(C)*	Structural characteristics of triglycerides	Villanova, Pa.	No	
E6 3-60(C)*	Dielectric properties of fatty peroxides	Philadelphia, Pa.	No	
E6 3-61(C)	Substituted vinyl monomers and polymers	Tucson, Arizona	Yes	4-B-1
E6 3-62	Synthetic lubricants from animal fats	Wyndmoor, Pa.	Yes	4-B-2
E6 3-63	Organic-inorganic fat compounds for use in plastics	Wyndmoor, Pa.	Yes	4-B-1
E6 3-64	Structure of components and derivatives of animal fats	Wyndmoor, Pa.		
E6 3-65	Fractionation and analysis of lipids	Wyndmoor, Pa.	Yes	4-A-1
E6 3-66	Polymerizable amides from animal fats	Wyndmoor, Pa.	Yes	4-B-1
E6 3-67(C)	Structural characteristics of organic peroxides	Pittsburgh, Pa.	Yes	4-D-2
E6 3-68	Autoxidation of fatty materials in emulsion	Wyndmoor, Pa.	Yes	4-A-2
E6 3-69 (C)**	Spatial interrelations within triglyceride molecules	Villanova, Pa.	No	
E6 3-69 (C)(Rev.)***	Spatial interrelations within triglyceride molecules	Villanova, Pa.	No	
E6 3-70 (C)***	X-ray investigation of mixed triglyceride	Villanova, Pa.	No	
E6 3-71 (C)***	Interfacial adsorption characteristics of salts of alkyl esters of α-sulfo fatty acids as related to their wetting and detergent action	Bethlehem, Pa.	No	
E6 3-72***	Development of industrially useful chemicals by free radical addition reactions	Wyndmoor, Pa.	Yes	4-B-2
E6 3-73***	Biodegradable detergents from animal fats	Wyndmoor, Pa.	Yes	4-C-1
E6 3-74 (Gr)***	Synthesis of pure model triglycerides	Not yet determined	No	
E6 3-75 (Gr)***	Ozonization of animal fats	Not yet determined	No	
UR-E9-(60)-79	Hydroxylated fatty derivatives	Marseilles, France	Yes	4-B-1
UR-E25-(60)-22	Cocoa butter substitutes from animal fats	Madrid, Spain	No	
E6 3-76***	New mathematical approaches to physical measurements	Wyndmoor, Pa.	No	
UR-A7-(60)-72***	Preparation and properties of long chain sulfated monoglycerides	Bombay, India		

* Discontinued during report period.
** Superseded by E6 3-69(C)(Rev.)
*** Initiated during report period.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
E6 4	Hides, Skins and Leather Utilization Investigations Program Leadership			
E6 4-26*	Reversible shrinkage in leather	Wyndmoor, Pa.	No	
E6 4-28(C)*	Conversion of dialdehyde tanned skins to salable light leather	Lowell, Mass.	No	
E6 4-29*	Improved water repellency for leather	Wyndmoor, Pa.	Yes	5-B-1
E6 4-31(C)*	Dynamic mechanical tester	Philadelphia,Pa.	No	
E6 4-32	Composition and properties of animal protein residues	Wyndmoor, Pa.	Yes	5-D-1
E6 4-33(C)*	Evaluation of collagen fibers from enzyme unhaired and lime-unhaired hides	Kansas City, Mo.	No	
E6 4-34	Processes for making commercial quality leather from enzyme-unhaired hides	Wyndmoor, Pa.	Yes	5-C-1
E6 4-35	New tanning processes for producing leathers of superior durability	Wyndmoor, Pa.	Yes	5-C-3
E6 4-36	Effect of electrolytes and lipid components on hide properties	Wyndmoor, Pa.	Yes	5-A-2
E6 4-37(C)	Noncollagenous proteins of cattlehides	Cincinnati, Ohio	Yes	5-A-3
E6 4-38(C)	Preparation and properties of dispersed collagen sols	Not yet determined		
E6 4-39	Microscopic investigation of skin and leather structure	Wyndmoor, Pa.	Yes	5-A-1
E6 4-40(C)	Abnormalities of leather characterized by a depleted mush texture	Cincinnati, Ohio	No	
E6 4-41	Physical properties of collagen and leathers	Wyndmoor, Pa.	Yes	5-A-1
E6 4-42	Addition of new reactive sites to hide proteins	Wyndmoor, Pa.	Yes	5-B-2
E6 4-43**	Chemical modification of hides with aldehydes in combination with phenols, amides, hydrazides	Wyndmoor, Pa.	Yes	5-B-2
E6 4-44**	Chemical modification of animal hides with cyclic urea derivatives such as urons and triazones	Wyndmoor, Pa.	Yes	5-B-2
E6 4-45 (Gr)**	Physical properties of collagen	Evanston, Ill.	No	
UR-A7-(60)-17	Polyphenolic tanning compounds	Madras, India	Yes	5-A-1
UR-A7-(60)-18	Relation of hide quality to tanning rate	Madras, India	Yes	5-C-3
UR-E8-(60)-3	Fractionation of gelatin and collagen	Turku, Finland	Yes	5-A-1
UR-E15-(60)-5	Microbial damage to exported U. S. hides	Naples, Italy	Yes	5-C-4
UR-E15-(60)-7	Tanning studies on American hides	Naples, Italy	Yes	5-C-5
UR-E29-(60)-2*	Deterioration of leather by sweat and heat	Surrey, England	Yes	5-C-2
UR-A7-(60)-43**	Hydrothermal shrinkage of collagen and leather	Madras, India	No	
UR-E19-(60)-13**	Kinetics of chrome tanning	Waalwijk, Holland	No	
UR-E29-(60)-67**	Chemically reactive compounds for improving leather stability	Surrey, England	No.	

* Discontinued during report period.

** Initiated during report period.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
E3 6	Potato and Other Vegetable Utilizations - Eastern Region. Program Leadership	Wyndmoor, Pa.		
E3 6-37*	Development of "instantized" dehydrated potato pieces	Wyndmoor, Pa.	Yes	6-B-1
E3 6-42	Nitrogenous constituents as quality factors in potato processing	Wyndmoor, Pa.	Yes	6-A-1
E3 6-43*	Improvement in storage stability of potato flakes and flakelets	Wyndmoor, Pa.	Yes	6-B-2
E3 6-44	Basic composition studies on the lipid fraction of potatoes	Wyndmoor, Pa.	Yes	6-A-3
E3 6-45	Basic studies on the formation and identity of the after-cooking discoloration pigment	Wyndmoor, Pa.	Yes	6-A-2
E3 6-46**	Color and texture of frozen french fried potatoes	Wyndmoor, Pa.	Yes	6-C-1
E3 6-47**	Effect of varietal, cultural and other source factors on the quality of processed potato products	E. Grand Forks, Minn.	Yes	6-C-2
E3 6	Potato and Other Vegetable Utilizations - Eastern Region. Program Leadership.	Wyndmoor, Pa.		
E3 6-41	Development of new types of dehydrated vegetables through modification of internal structure	Wyndmoor, Pa.	Yes	7-A-1; 7-B-1

* Discontinued during report year.

** Initiated during report year.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
E3 3	Apples and Other Fruit Utilization Investigations - Eastern Region. Program Leadership	Wyndmoor, Pa.		
E3 3-30	Development of improved apple cider	Wyndmoor, Pa.	Yes	8-B-2
E3 3-32	Rapidly-reconstitutable dried fruit products	Wyndmoor, Pa.	Yes	8-B-1 8-C-2
E3 3-33(C)	Relation of physical and chemical properties to processing characteristics of eastern peaches	New Brunswick, N. J.	Yes	8-C-1
E3 3-34	Improvement of processed cherries through studies on composition and post-harvest treatments	Wyndmoor, Pa.	Yes	8-A-1
E3 3-35(C)	Relation of apple cell wall constituents to textural quality of processed products	College Park, Md.	Yes	8-A-2
E5 3	Tobacco Investigations. Program Leadership	Wyndmoor, Pa.		
E5 3-5	Acids and bases in cigar smoke	Wyndmoor, Pa.	Yes	9-A
E5 3-6	Composition of cigarette smoke	Wyndmoor, Pa.	Yes	9-A
E5 3-7	Composition of oxidation products	Wyndmoor, Pa.	Yes	9-B
E5 3-8 (C)(Rev.)*	Investigations of neutral resins	Durham, N. C.	No	- -

* Initiated during report period. Supersedes E5 3-8(C) and E5 3-8(Gr) which were not activated.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
E5 1	Sugars and Sirups Investigations. Program Leadership	Wyndmoor, Pa.		
E5 1-67(C)	Improvement in processing and quality by study of factors affecting sugar sand formation	Wooster, Ohio	Yes	10-A-1
E5 1-74	Chemical compounds responsible for maple flavors; precursors of maple flavor	Wyndmoor, Pa.	Yes	10-B-1
E5 1-76	Improvement of the quality of maple products through a study of the fermentation-induced biochemical reactions involved in the formation of maple color and flavor	Wyndmoor, Pa.	Yes	10-C-1
E5 1-78(C)*	Improvement of the maple industry through development of methods for prolonged storage of maple sap: Control of microbial fermentation of sap before and after delivery to storage tanks	Undetermined	No	
E5 1	Sugar and Sirups Investigations. Program Leadership	Wyndmoor, Pa.		
E5 1-68	Isolation, characterization and properties of honey enzymes	Wyndmoor, Pa.	Yes	11-A

* Initiated during report period.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
E5 5	New and Replacement Crops Utilization Investigations. Program Leadership.	Wyndmoor, Pa.		
E5 5-31*	Development of plant sources of precursors for steroid hormones: assay of agronomic samples for steroidal sapogenins.	Wyndmoor, Pa.	No	
E5 5-39	New crop seed epoxy-containing oils.	Wyndmoor, Pa.	Yes	12-A

*Discontinued during report year.